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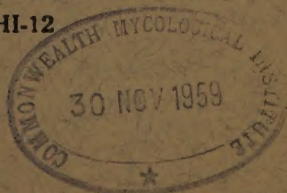
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# ELECTRON MICROSCOPICAL STUDIES OF ULTRA-THIN SECTIONS IN *PENICILLIUM CHRYSOGENUM*

## III. THE FINE STRUCTURE OF CYTOPLASMIC GRANULES\*

SEIZO TSUDA

(Accepted for publication September 1, 1957)

**INTRODUCTION.** It has been reported in the previous paper (Tsuda, 1955, 1956) that the mycelium of *Penicillium* is wrapped in a single layer of apparently homogeneous cell wall, while the nucleus as well as the cytoplasm appear to be of a loose filamentous structure, many cytoplasmic granules being scattered in the cytoplasm.

Cytoplasmic granules in fungi have been studied by many authors genetically, cytologically as well as biochemically. The present author has found that the cytoplasmic granules in *Penicillium chrysogenum* are heavily stained by the Janus green B under the light microscope, though the data have not been published. This staining reaction seems to suggest that these granules may have a respirational function. The granules in *Penicillium* may be substantially the same as those described by Ephrussi (1951), Mundkur (1953), Hartman and Liu (1954) in yeast, and by Mitchell *et al* (1953) in *Neurospora*.

The purpose of this paper is to give some additional information on the fine structure of these cytoplasmic granules as obtained by the study of ultra-thin sections.

**MATERIALS AND METHODS.** *Penicillium chrysogenum* was cultivated in submerged condition, and the cultivated material was precipitated by moderate centrifugation. The material was fixed in 0.5 per cent osmium tetroxide in phosphate-buffered solution for 30 minutes, washed several times with distilled water, then dehydrated through a graded ethyl alcohol series, and were finally imbedded in a mixture of 95 parts of n-butyl methacrylate and 5 parts of methyl methacrylate with 2 per cent benzoyl peroxide as catalyst.

The material in this plastic substance was cut into thin sections by ultramicrotome, and the specimens were examined under a J. E. M. type III electron microscope made by Japan Electron Optics Laboratory.

**RESULTS AND DISCUSSION.** The finding of reduced number of cytoplasmic granules in the mycelium of *Penicillium* is in agreement with the results of the study of ultra-thin sections by electron microscope.

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\*Contributions from the National Institute of Genetics, Japan, No. 856

As may be seen from figure 1, the granules are usually grouped in certain areas of the cytoplasm. When they are cut transversely or obliquely they show a system of internal ridges which are attached to the granular membrane which in turn is filled with a relatively homogeneous material. A great variation in the diameter of the cytoplasmic granules of the mycelium was observed, ranging in size from 0.61 to 0.91 $\mu$ . The number in some cells was several times that in other cells.

The granules are surrounded by a defined membrane as the photographs show. The inner layer of the membrane projects at intervals into the interior of the granules. No pores were observed in the membrane.

As shown in figure 2, the circular or oval form of the profiles indicate that the granules were cut transversely. Under such circumstances the internal ridges are viewed from the side and appear to have a radial arrangement. Figure 3 shows longitudinal and transverse sections of granules. At the left end of the micrograph is seen longitudinal section showing a number of internal ridges. Further to the right in the transverse sections "cristae" may be seen projecting into the structureless matrix. The thickness of the granular membrane varies from about 20 to 40 m $\mu$ . These variations in thickness indicate that the granules are cut at various angles; if the sections are cut at right angle to the surface of the granule the membrane looks thin, while sections through the membrane at angles of increasing obliqueness produce wider bands.

A large number of cytoplasmic components considered to be degenerate granules were seen in old culture materials. As seen from figure 4, their inner structure has become a network, due to many vacuoles in the cytoplasm. The limiting membrane, however, could be seen clearly at some points.

For a long time the structure of cytoplasmic granules has been of particular interest to the microbiologist and biochemist. In the present study on *Penicillium*, the fine structure of cytoplasmic mitochondria-like granules possessing a limiting membrane and showing a system of internal ridges was confirmed.

It is assumed that those granules may have the enzymatic activity. This point awaits further investigation.

**ACKNOWLEDGMENT.** The author wishes to express his gratitude to Dr. M. Tsujita for his valuable suggestions and criticisms.

**SUMMARY.** The present paper deals with some additional information on the fine structures of the cytoplasmic granules of *Penicillium chrysogenum* obtained from ultra-thin sections.

The granules were mostly spherical or oval. The fine structure of the granules possessing a limiting membrane and showing a system of internal ridges was confirmed.



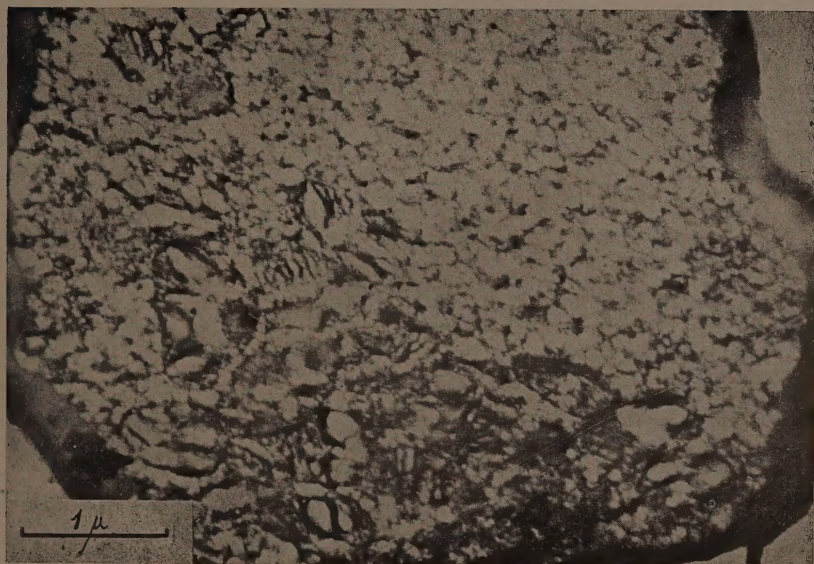


Fig. 1.



Fig. 2

Figure 1. Cytoplasmic granules can be seen grouped in certain areas of the cytoplasm. The granules cut transversely or obliquely show in this electron micrograph a system of internal ridges.

Figure 2. Transverse sections of cytoplasmic granules. The granular profiles are of circular or oval form.



Fig. 3

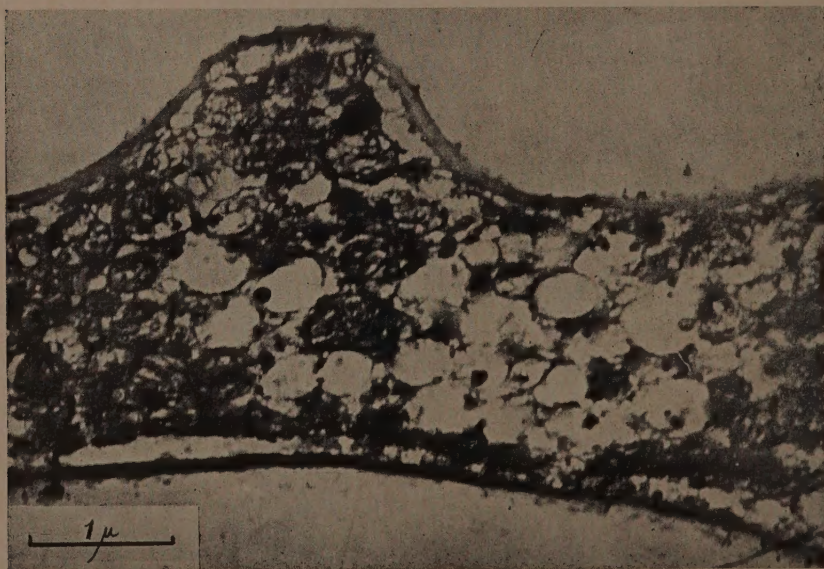


Fig. 4

Figure 3. Longitudinal and transverse sections of cytoplasmic granules. Systems of internal ridges viewed from the side appear to have various shapes.

At the left end of the profile where the section is cut parallel to the long axis a number of internal ridges can be seen.

Figure 4. A large number of cytoplasmic components considered to be degenerate cytoplasmic granules are shown in this electron micrograph.



A great variation in the diameter of the cytoplasmic granules of the mycelium was observed, ranging in size from 0.61 to 0.91 $\mu$ . The number of the granules in some cells was several times that in other cells.

A large number of cytoplasmic component-considered to be degenerate granules were seen in old culture materials.

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## THE MYXOMYCETES OF THE MUSSOORIE HILLS - VII

K. S. THIND AND P. S. REHILL

(Accepted for publication September 10, 1957)

The first six contributions (listed under references) describe 37 known species, mostly new records in India, and 3 new species of Myxomycetes. This seventh contribution deals with 7 more species and one atypical form collected from the same region. Out of these, two species, one variety and one atypical form - *Ceratiomyxa fruticulosa* (Müll.) Macbr. var. *arbuscula* Berk. & Br., *Fuligo cinerea* (Schw.) Morgan, *Physarum didermoides* (pers.) Rost., and atypical form of *Physarum echinosporum* Lister are more new records in India.

The classification of Martin (1949) has been followed in the previous as well as in the present study.

The numbers of the species are the serial numbers of the Myxomycetous Flora of the Mussoorie Hills.

Type collections have been deposited in the Herbarium of the Punjab University. Duplicate material is in the State University of Iowa, U.S.A.

The authors are deeply indebted to Dr. G. W. Martin of the State University of Iowa, U.S.A. for help in the determination of the species, and Prof. P. N. Mehra, Head of the Panjab University Botany Department, for providing facilities and encouragement. They are also thankful to Mr. B. Khanna for making illustrations of the fruit bodies.

Sub-class: *Exosporeae*

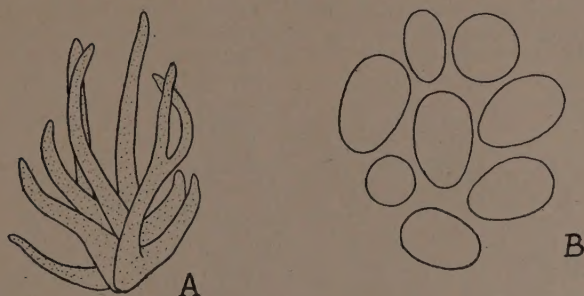
Order: *Ceratiomyzales*

### 41. *Ceratiomyxa fruticulosa* (Müll.) Macbr.

*Sporophores* snow white to whitish, gregarious, mould-like patches on the substratum, arising in clusters (up to 6 in a cluster) of erect pillars from broadly effused hypothallus, clusters 0.5 - 2.5 mm. tall and up to 2 mm. broad: each pillar or a sporophore usually simple, or sparsely branched (only once or rarely twice), up to 0.37 mm. wide, narrowed upward or not, apices obtuse, densely covered all over with stalks which bear spores singly: stalks like sterigmata of basidia, hyaline, very fine and thread-like, tapering above to a fine point, up to 10 $\mu$  long.

*Spores* borne externally on slender, uniform stalks, white in a mass, hyaline under the microscope, variable in shape and size, predominantly oval or ellipsoid, some times globose to sub-globose, smooth, 7- 10 x 6-8  $\mu$ , mostly 9 x 7.5  $\mu$ .





Text-Fig. 1. *Ceratiomyxa fruticulosa* (Müll.) Macbr. A Sporophores of n. 105 arising in a cluster of erect pillars,  $\times 20$ . B. Predominantly ellipsoid spores of n. 105,  $\times 1150$ .

Collected on dead leaves of *Agave* sp. and on a living moss growing on bark of a tree, Nala Pani, Dehra Dun, August 7, 1954, 105. On decaying wood of a tree, Chakrata Toll, Mussoorie, July 16, 1954, 106.

Both the collections, n. 105 and 106 are typical of the species and are characterized by white fruit bodies springing in clusters and simple to sparsely branched, and mostly broadly oval spores measuring mostly  $9 \times 7.5 \mu$ . This fungus appears to be quite common in the Mussoorie Hills and presents a striking mold-like appearance on the substratum.

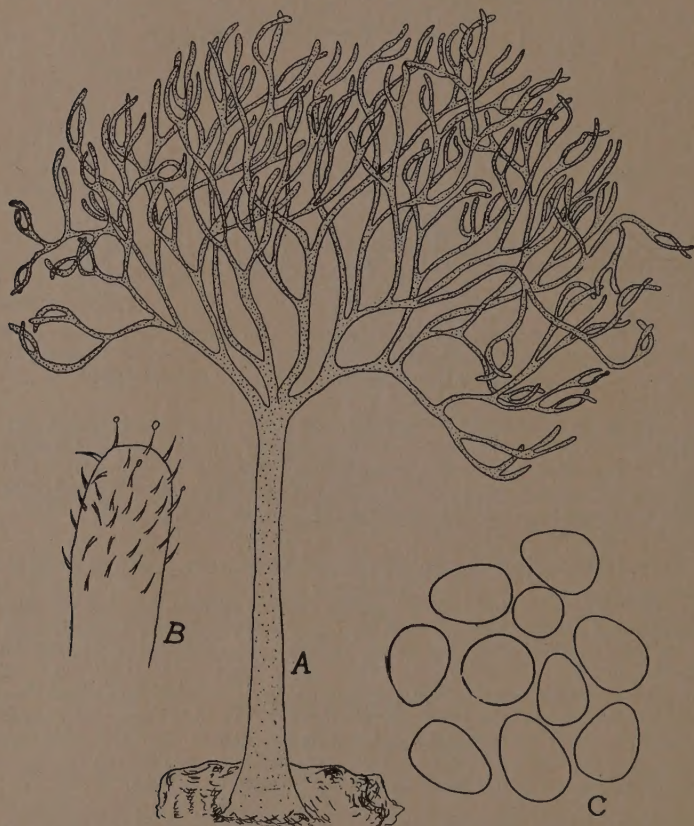
42. *Ceratiomyxa fruticulosa* (Müll.) Macbr. var. *arbuscula* Berk. & Br.

*Sporophores* appearing as snow white, mould-like, large, densely gregarious patches extending up to 7.5 cm. over the substratum, up to 8 mm. tall, stalked or with a trunk which becomes profusely branched in tree-like fashion: trunk up to 5 mm. long and up to 0.37 mm. wide: branching mostly dichotomous, coralloid and tree-like, branches thinner than the trunk, do not taper, overlapping, often entangled with those of the neighbouring sporophores: apices obtuse, may or may not be enlarged: stalks densely covering whole of the sporophore including the trunk, very fine, tapering to a fine point at the top, up to  $10 \mu$  long.

Collected on dead wood of a tree, Saharanpur Road, Dehra Dun, August 4, 1954, 107. New record in India.

The collection n. 107 is easily recognized from n. 105 and n. 106 by its sporophores branching in a tree-like fashion at the top of the trunk. It is regarded here as var. *arbuscula* Berk. & Br. with which it resembles closely. In doing so we have followed the old custom because the recent trend is not to recognize any form or variety for *C. fruticulosa* which, otherwise, is an extremely variable species (Martin, 1949). All the various varieties proposed for this species are reported to overlap considerably. Since no such overlapping is reported as yet from this country, it is considered desirable to recognise provisionally, atleast for the sake of convenience, varieties for this species whenever some big deviations are observed.

Spores borne singly on the stalk, white in a mass, hyaline under the microscope, mostly broadly oval or ellipsoid, with some intermixed globose and subglobose spores, smooth,  $6-8 \times 8-12 \mu$ , mostly  $9 \times 7.5 \mu$ .



Text-Fig. 2. *Ceratiomyxa fruticulosa* var. *arbuscula* Berk. & Br. A. A sporophore branching in a tree-like fashion at the top of its trunk, x 20. B. Apical part of a sporiferous branch covered all over with stalks bearing spores singly at the top, x 320. C. Predominantly ellipsoid spores, x 1150.

Sub-class *Endosporeae*

Order: *Physarales*

#### 43. *Fuligo septica* (L.) Weber.

Fructifications aethaliate. *Aethalia* occurring singly, variable in colour and size, usually large, white, cream coloured, yellowish, yellowish orange, or dull orange, pulvinate or cushion-like, depressed, globose, subglobose, or considerably elongated, covering small to very large surface of the sub-



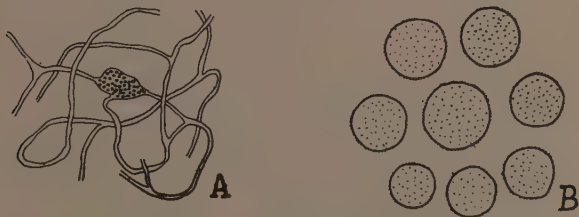
stratum, 1-8 cm. in diameter, or extending up to a maximum length of 21 cm., 0.5-1.5 cm. thick: hypothallus membranous, white, later turning yellowish brown, present all around the aethalium: cortex calcareous, very fragile, typically foamy, thick: dehiscence irregular, the highly fragile cortex falls off at maturity exposing the spore masses, cortex disappearing from top of the aethalium downward: central sporiferous region composed of fused sporangia or more probably plasmodiocarps which are marked off by the calcareous, tortuous, anastomosed, very fragile walls. These calcareous walls or partitions are regarded by Macbride and Martin (1934) as forming pseudo-capillitium in addition to the true capillitium.

*Columella* absent.

*Capillitium* abundant, extending within the sporangial or plasmodiocarpous walls, chiefly composed of the non-calcareous, hyaline, slender, forking and rarely anastomosing thread-like internodal tubules: nodes rare or scanty, small, fusiform, calcareous, pale yellow, interconnected by internodal threads.

*Spores* black in a mass, purplish violet under the microscope, globose to subglobose, inconspicuously verrucose, 6-9  $\mu$  in diameter.

*Plasmodium* reddish brown.



Text-Fig. 3. *Fuligo septica*, A. Forking and rarely anastomosing slender internodal tubules with a small and fusiform node,  $\times 320$ . B. Inconspicuously verrucose small spores,  $\times 1115$ .

Collected on organic debris, Mossy Fall, Mussoorie, July 22, 1954, 108. On dead bark of a tree log, Hardwar Road, Dehra Dun, August 12, 1954, 109.

These two collections undoubtedly belong to *Fuligo septica* (L.) Webber. The species has been collected abundantly from the Mussoorie Hills and Dehra Dun Valley and shows a great variation of colour and size as is true of it.

The nodular thickenings in the capillitium of these collections are rare to scanty but these are reported to be usually well represented in *F. septica*.

A collection of *Fuligo septica* Gmelin reported from Madras (Agnihothru, 1954) evidently possesses abundant lime knots in its capitalitium.

This collection, however, represents only the much smaller aethalium. It may also be pointed out here that the authority of Gmelin is no longer recognized for this species which should be correctly written with the authority as done here. Agnihotrudu has apparently followed the nomenclature of Lister, 1925, which is not accepted by later workers.

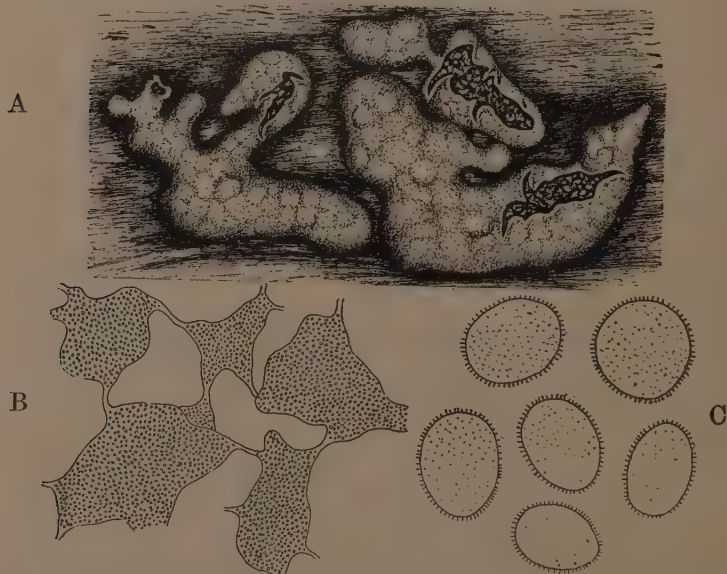
44. *Fuligo cinerea* (Schw.) Morgan

*Fructifications* aethaloid to subplasmodiocarpous: aethalia pulvinate, elongate, simple or branched, small, scattered or gregarious, often heaped together into bigger masses and hence appearing lobate, up to 22 mm. long, 2-5 mm. wide and up to 2 mm. high, white, formed of closely interwoven sporangia as indicated by the presence of calcareous walls or partitions in an exposed aethalium; hypothallus profusely developed, cream coloured, ridged: peridium or cortex thick, single, white, firm, crustose, heavily calcareous: dehiscence irregular.

*Columella* none.

*Capillitium* well developed, white, composed of nodes and internodes: nodes calcareous, abundant, small to large, spherical to elongated or fusiform, irregular in size and shape: internodes hyaline, slender noncalcareous.

*Spores* black in a mass, brownish purple under the microscope, predominantly broadly elliptical, some globose to subglobose, distinctly and prominently verrucose, 12-15  $\times$  11-12  $\mu$  when ellipsoid and 9-13  $\mu$  in diameter when globose.



Text-fig. 4. *Fuligo cinerea* (Schw.) Morgan. A. Aethaloid to subplasmodiocarpous fructifications,  $\times$  5. B. Capillitium,  $\times$  320. C. Prominently verrucose spores,  $\times$  1150.



Collected on dead leaves of *Dalbergia sissoo* Roxb., *Saccharum* sp., and on rotten cloth pieces, Doiwala, Dehra Dun, August 25, 1953, 110.

This fungus undoubtedly belongs to *Fuligo cinerea* (Schw.) Morgan and is characterized by small aethaloid fructifications enclosed by a white crust and predominantly elliptical spores.

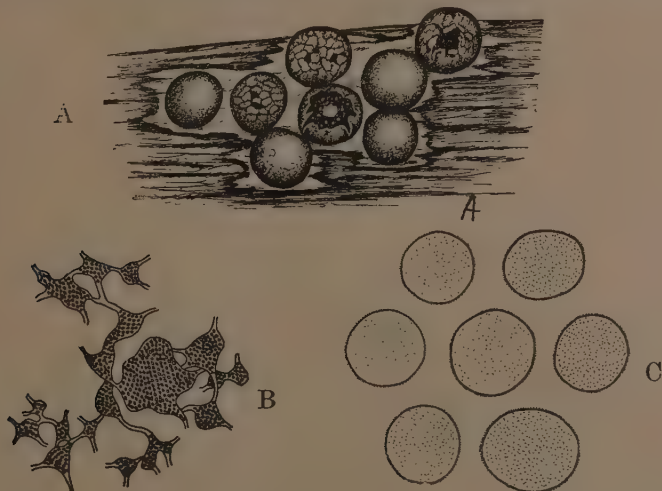
45. *Physarum Didermoides* (Pers.) Rost.

*Fructifications* sporangiate: sporangia densely crowded forming a sporangial crust over the substratum up to 5 cm. long, often connate, sessile, globose to subglobose to pulvinate, often angular due to mutual compression, grayish white changing to dark gray due to the falling of outer calcareous deposits, 0.42 – 0.64 mm. in diameter: hypothallus profusely developed, effuse or confluent, white, calcareous: peridium plainly double: outer peridium white, calcareous, crustose, breaking into white, more or less circular plates: inner peridium membranous, dark coloured, dark brown inside: dehiscence irregular but usually at the top especially in the case of connate sporangia.

*Columella* none.

*Capillitium* profusely developed, white, composed of nodes and internodes: nodes numerous, small, spherical to angular, calcareous, often massed together in the centre to form a conspicuous pseudocolumella, interconnected either directly or through hyaline, branched, noncalcareous, slender threads.

*Spores* black in a mass, dark violet under the microscope, globose, profusely and minutely verrucose, uniguttulate, 11.2 – 13.6  $\mu$  in diameter.



Text-Fig. 5. *Physarum Didermoides* (Pers.) Rost. A. Fructifications  $\times 20$ . B. Capillitium,  $\times 130$  C. Minutely verrucose spores,  $\times 1150$ .

Collected on dead bark of a tree, Dehra Dun, August 4, 1953, 111. On dead and decaying leaves of *Agave* sp., Adunca Bridge, Mussoorie, September 8, 1956, 112.

This interesting fungus seems to be quite common in the Mussoorie Hills and is characterized by densely gregarious, sessile sporangia, outer crustose peridium, and dark coloured spores.

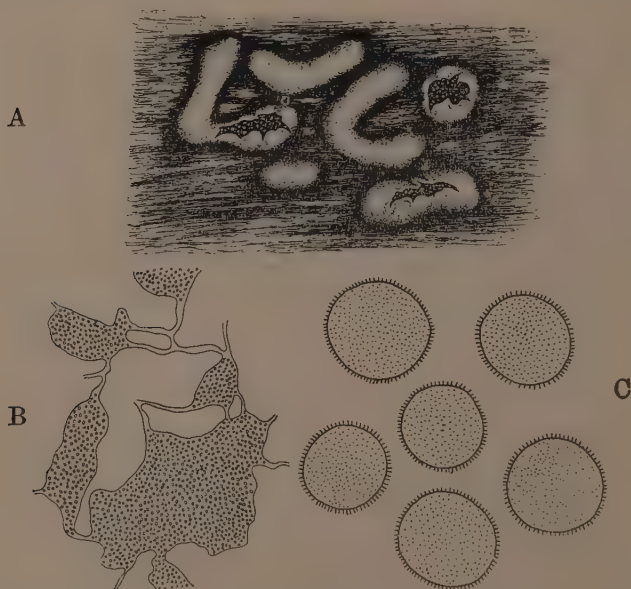
46. *Physarum bitectum* G. Lister

*Fructifications* mostly plasmodiocarpous with some sporangial types, gregarious, jet black, smooth, lustrous and iridescent: plasmodiocarps short to long, terete, mostly simple, rarely branched, straight to flexuous, rarely arcuate, up to 3 mm. long and up to 0.4 mm. wide, sometimes reduced to globose or subglobose, sessile sporangia, 0.25 – 0.38 mm. in diameter: hypothallus absent: peridium single, thin, membranous, jet black, iridescent, noncalcareous, slightly wrinkled at the top: dehiscence irregular.

*Columella* absent.

*Capillitium* abundant, composed of nodes and internodes: nodes white, large and often massed in the centre, calcareous, irregular in shape and size, interconnected by slender, hyaline, noncalcareous, branched internodes.

*Spores* black in a mass, dark brown to almost black under the microscope, globose to subglobose, prominently and profusely verrucose, 11–14  $\mu$  (mostly 10–12  $\mu$ ) in diameter.



Text-Fig. 6. *Physarum bitectum* G. Lister, A. Fructifications,  $\times 20$ . B. Capillitium,  $\times 320$ . C. Prominently verrucose spores,  $\times 1150$ .



Collected on decaying leaves of *Agave* sp., Jumna Bridge, Mussoorie, August 27, 1953, 113.

This Mussoorie collection is an interesting phase of *Physarum bitectum* G. Lister in which the outer peridium has failed to form the usual, white, calcareous, firm, outer crust and is represented only by the persistent, jet black, iridescent, noncalcareous, inner peridium. The very dense capillitium with large, irregular nodes, often massed in the centre, and the dark, warted spores, mostly 10–12  $\mu$  in diameter, are characteristic of *Physarum bitectum*.

#### 47. *Physarum echinosporum* Lister

*Fructifications* plasmodiocarpous, rarely with sporangial types: plasmodiocarps gregarious, dull white or chalk white, strongly compressed laterally, short to long, straight to bent to arcuate, mostly simple, rarely branched, up to 5 mm. long and 0.55–1 mm. in height: hypothallus absent: peridium plainly double: outer peridium thick, egg-shell like, brittle, white, calcareous with spherical granules: inner peridium distinct from the outer one, thin, membranous, fragile, gray iridescent: dehiscence irregularly longitudinal, the outer peridium rupturing irregularly at the summit.

*Columella* absent.

*Capillitium* dense, composed of nodes and internodes: nodes white, large, often massed in the centre, spherical to elongated, calcareous, interconnected by poorly developed, hyaline, short, noncalcareous internodes, sometimes nodes connected directly.

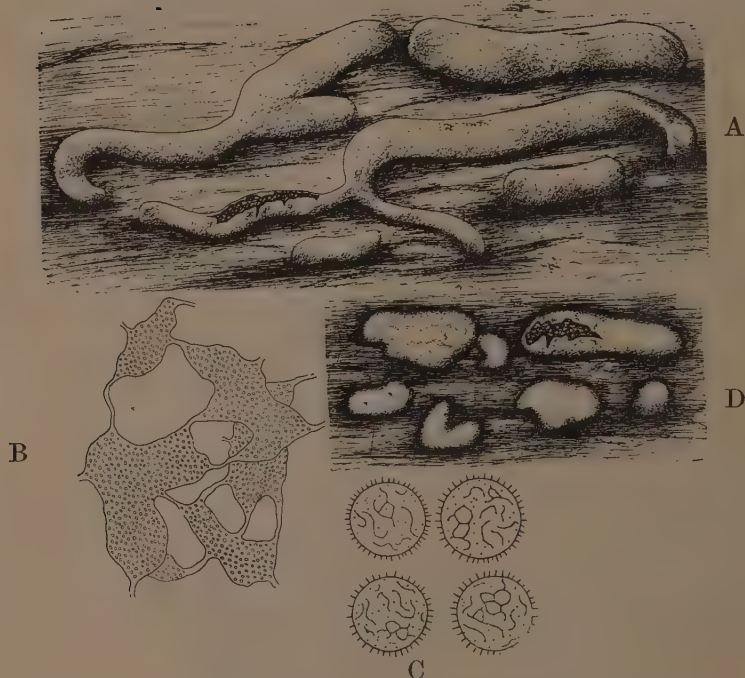
*Spores* black in a mass, dark brown or dark violet under the microscope, globose to subglobose, prominently and profusely verrucose, warts interconnected to form a reticulation, 8.8–12  $\mu$  in diameter.

Collected on dead leaves of Oak and several other plants, Doiwala, Dehra Dun, August 9, 1953, 114. (Text fig. 7, A–C)

#### 48. *Physarum echinosporum* Lister.

“An atypical form”.

*Fructifications* mostly plasmodiocarpous, with a few sporangial types, gregarious, chalk white: plasmodiocarps mostly short, sometimes long, straight to flexuous, terete or laterally to transversely compressed, simple, up to 7.5 mm. long and 0.5–1.3 mm. wide: sporangia pulvinate, globose to subglobose, not laterally compressed: hypothallus absent: peridium double: dehiscence irregularly longitudinal: *Columella* absent: capillitium dense, nodes often massed together in the centre: spores dark violet, globose to subglobose, profusely and prominently verrucose, warts interconnected to form a reticulation, 8–14  $\mu$  in diameter.



Text-Fig. 7. *Physarum echinosporum* Lister, A. Strongly and laterally compressed fructifications, x 10. B. Capillitium, x 320. C. Prominently verrucose spores with incomplete reticulations, x 1150. D. *P. echinosporum* "An atypical form" Terete or laterally to transversely compressed fructifications, x 10.

Collected on dead leaves and dead twigs, New Forest, Dehra Dun, August 22, 1953, 115. (Text fig. 7, D)

This collection n. 115 is an atypical form and shows very irregular development. Its plasmodiocarps are cylindrical or laterally to transversely compressed while its sporangia are pulvinate and not laterally flattened. Its spores are irregular in shape and size and many erupted. This suggests premature drying while fruiting followed by a soaking.

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Panjab University, Amritsar.



Fig. 1

Fig. 2

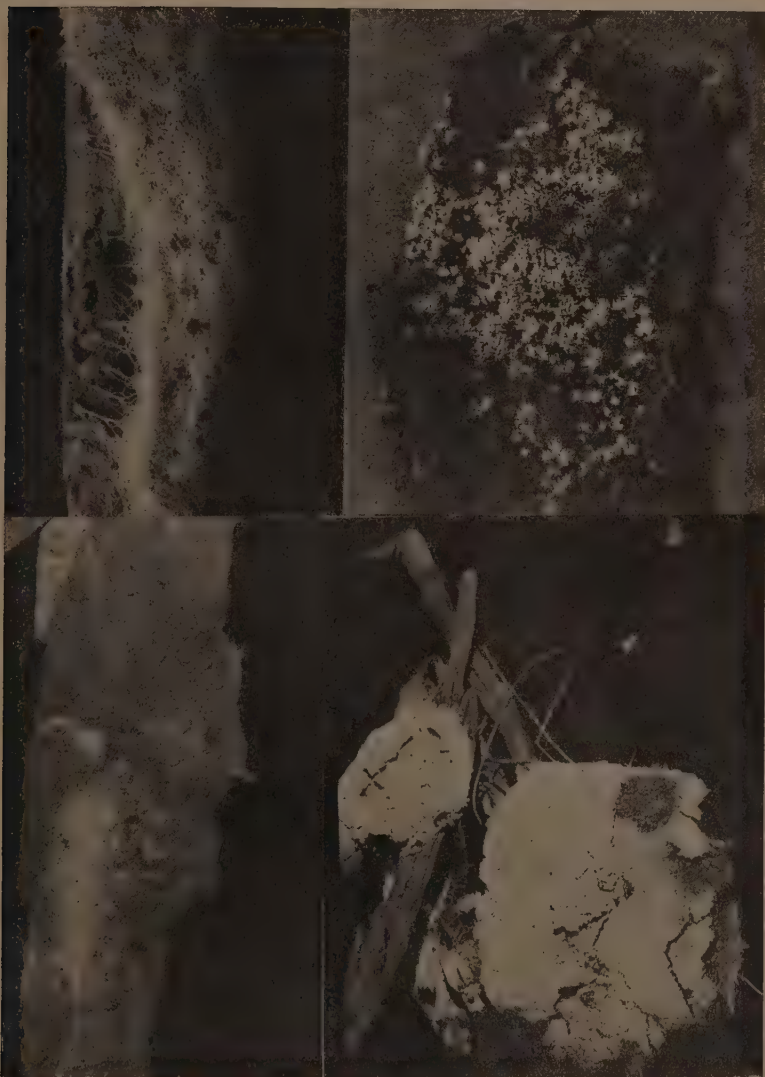


Fig. 3 (b)

Fig. 3 (a)

1. *Ceratiomyxa fruticulosa*, n. 105,  $\times 1$  (approx.)
2. *Ceratiomyxa fruticulosa* var. *arbuscula*,  $\times 3$  (approx.).
3. *Fuligo septica*, (a) pulvinate aethalia; (b) a considerably elongated aethalium,  $\times \frac{1}{2}$  (approx.).

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## THE MYXOMYCETES OF THE MUSSOORIE HILLS - VIII

K. S. THIND and M. S. MANOCHA

(Accepted for publication September 20, 1957)

The first seven contributions (listed under references) describe 44 known species, one known variety and 3 new species of Myxomycetes. This eighth contribution deals with 7 more known species of which 5 are new records for India.

The classification of Martin (1949) has been followed throughout this study, although monograph of Lister and Lister (1925) and Macbride and Martin (1934) were freely consulted.

The numbers of the species are the serial numbers of the myxomycetous flora of the Mussoorie Hills.

Type collections have been deposited in the Herbarium of the Punjab University and Herbarium Crypt. Ind. Orient, New Delhi.

The authors are deeply indebted to Prof. P. N. Mehra, Head of the Panjab University Botany Department, for encouragement and providing facilities, and also thankful to Mr. B. Khanna for drawing the illustrations of the fructifications.

### 49. *Physarum vernum* Somm. ex. Fries

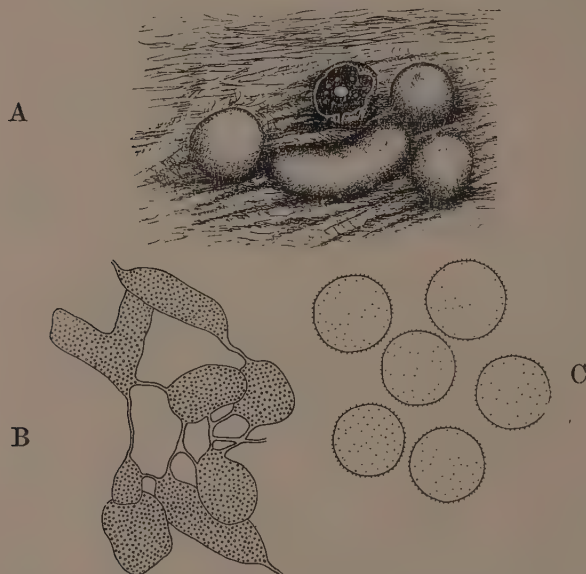
*Fructifications* sporangiate to plasmodiocarpous, the former predominating: sporangia densely gregarious, sessile, narrowed below into a stem-like base, whitish gray or ashen coloured (or cinereous), globose to subglobose, 0.4-0.5 mm. in diameter: plasmodiocarps small, simple, straight or curved, concolorous with the sporangia, 0.4 mm. in diameter, up to 1 mm. long: hypothallus not observed: peridium single, thin, membranous, iridescent with purple-blue reflections, covered over densely with white cottony, spherical to irregular flakes of lime, lime flakes falling off with age: dehiscence irregular.

*Columella* absent.

*Capillitium* dense, composed of profusely developed nodes and poorly developed internodes: nodes abundant, large, white, calcareous, calcareous matter composed of spherical granules, spherical to angular or irregular, variable in shape and size, aggregated in the centre to form a prominent pseudocollumella, often joined with one another (without an internode): internodes hyaline short, slender, noncalcareous, inconspicuous or appearing masked by the profusely developed nodes.



*Spores* black in a mass, dark-violet under the microscope, globose, prominently verrucose, warts narrow and spine like, 11.2–12.4  $\mu$  in diameter.



Text-Fig. 1. *Physarum vernum* Somm. ex Fr., A. Sporangiate to plasmodiocarpous fructifications,  $\times 20$ . B. Capillitium with profusely developed nodes and poorly developed internodes,  $\times 400$ . C. Prominently verrucose spores,  $\times 1000$ .

Collected on dead leaves of *Agave* species, Jamna Bridge, Mussoorie, September 8, 1956, 164.

This collection undoubtedly belongs to *Physarum vernum* Somm. ex Fries and is characterized by the profusely developed nodes often forming a pseudocolumella in the centre of fruit bodies and dark coloured, prominently verrucose, large, spores. In these respects it is easily differentiated from the closely allied *Physarum cinereum* (Batsch) Pers.

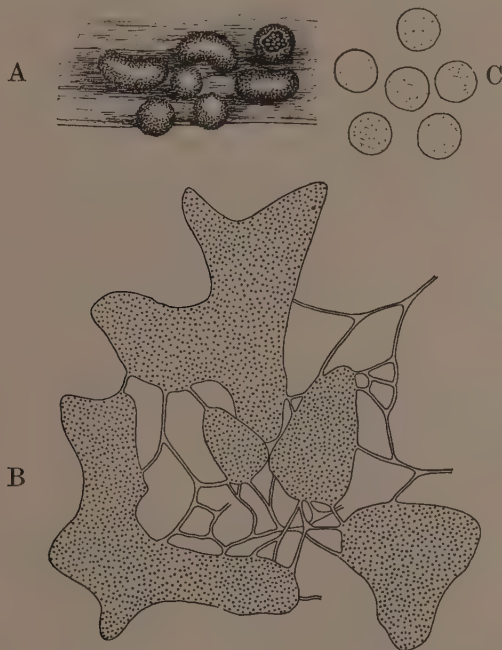
#### 50. *Physarum lateritium* (Berk. and Rav.) Morgan

*Fructifications* plasmodiocarpous to sporangiate types, densely gregarious, red to orange red, colour usually dull and fading with age: plasmodiocarps short, rough, unbranched, straight, flexuous, rarely arcuate, terete, never netted, up to 3 mm. long, up to 0.25 mm. wide: sporangia globose to subglobose, sessile, 0.25 – 0.4 mm. in diameter: hypothallus absent: peridium single, thin, covered over densely with red to orange red, nodular, calcareous deposits: dehiscence irregular: columella none.

*Capillitium* dense, composed of numerous nodes and profusely developed internodes: nodes large, pale yellow to pale orange, fading to white with

red centres in broken specimens, roundish to fusoid, calcareous : internodes hyaline, slender, branched, noncalcareous.

*Spores* black in a mass, violaceous brown under the microscope, globose to subglobose to ovoid, faintly verrucose to almost smooth,  $7-9\mu$  in diameter.



Text-Fig. 2. *Physarum lateritium* (Berk. & Rev.) Morgan, A. Plasmodiocarpous to sporangiate fructifications, with peridium covered over with nodular calcareous deposits,  $\times 20$ . B. Capillitium,  $\times 400$ . C. Faintly verrucose to almost smooth spores,  $\times 1000$ .

Collected on dead leaves of *Agave* species, Jamna Bridge, Mussoorie, September 10, 1953, 165. On dead roots and living twigs, Kempty Fall Road, Mussoorie, August 30, 1956, 166. New record in India.

This beautifully coloured species appears to be common in the Mussoorie Hills. It is characterized by reddish fruit bodies, pale coloured nodes of the capillitium fading to white with red centres and faintly verrucose spores. The Mussoorie collections are mostly plasmodiocarpous in contrast to the usually sporangial fruit bodies reported for this species.

#### 51. *Physarum mutabile* (Rost.) G. Lister

*Fructifications* mostly sporangiate with a few plasmodiocarps : sporangia densely gregarious, stipitate or sessile, whitish gray, yellowish

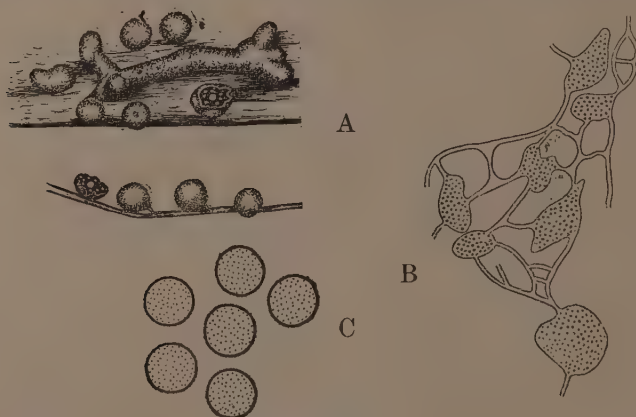
at the base, subglobose to oblong, sometimes obovate,  $0.34 - 0.48 \times 0.26 - 0.35$  mm.: stalk usually present, very small, limeless, yellowish, rugulose, up to 0.07 mm. long : plasmodiocarps short, up to 1.7 mm. long, simple to branched, straight to irregular: hypothallus small, yellowish, rotate: peridium single, thin, membranous, calcareous throughout, also with abundant squamulose lime deposits, lime composed of small round crystals: dehiscence irregular, basal portion persisting as a shallow cup.

*Columella* none.

*Pseudocolumella* present in the centre as calcareous white, usually elongate irregular mass.

*Capillitium* dense, intricate, persistent, composed of abundant nodes and internodes. Nodes white, calcareous, elongate, irregular in shape and size, massed together in the centre to form a pseudocolumella, interconnected by very fine, hyaline, noncalcareous internodes.

*Spores* black in a mass, purplish brown under the microscope, globose, profusely and minutely verrucose, warts minute but conspicuous and uniformly distributed,  $7.2 - 9.2 \mu$  in diameter, mostly  $8 \mu$  in diameter.



Text-Fig. 3. *Physarum mutabile* (Rost.) G. Lister, A. Sporangiate to plasmodiocarpous fructifications,  $\times 20$ . B. Capillitium,  $\times 400$ . C. Minutely verrucose spores,  $\times 1000$ .

Collected on dead leaves and dead pods of *Dalbergia sissoo* Roxb., on dead leaves of *Saccharum* species and on dead twigs, Dehra Dun, September 13, 1955, 167. New record for India.

This species was collected abundantly from Dehra Dun and is marked by white sporangia with intermixed short plasmodiocarps, yellow small stipe which is often absent altogether, squamulose deposits on the peridium, a pseudocolumella at the centre of fruit bodies, and minutely and profusely



verrucose spores (mostly 8  $\mu$  in diameter). It is easily differentiated from *Physarum nucleatum* Rex (Thind and Sohi, 1955) which possesses globose sporangia with long stalks.

### 52. *Physarum stellatum* (Masse) G. W. Martin

*Fructifications* sporangiate: sporangia densely gregarious, stipitate, gray or bronze coloured, white at the base, globose, erect to nodding, 0.37 – 0.52 mm. in diameter: stipe long, erect to bending, white, calcareous, subulate, tapering upward, longitudinally rugose, extending over the base of the sporangium as a circular white cap, 0.65 – 1 mm. long, i.e. twice the diameter of the sporangium: hypothallus prominent, calcareous, ridged, rotate peridium single, very thin and membranous, iridescent with a metallic lustre, covered over with white, discrete, globose to elongated, calcareous flakes or scales: dehiscence irregular, the peridium usually splitting in petaloid lobes.

*Columella* absent.

*Pseudocolumella* present in the form of conspicuous, small, white, spherical, calcareous mass.

*Capillitium* radiating from the central Pseudocolumella, not dense, reticulate, composed of nodes and internodes: nodes white, calcareous, more abundant near the pseudocolumella, scanty, in outer sporangial region, small, elongated to fusiform: internodes hyaline, short to long, noncalcareous.

*Spores* black in a mass, purple to purplish brown under the microscope, globose, very minutely verrucose, 8 – 10  $\mu$  in diameter.

Collected on dead wood and on mosses on Oak stump, The Park, Mussoorie, August 28, 1956, 168. New record in India.

This fungus undoubtedly belongs to *Physarum stellatum* (Masse) G. W. Martin and is characterized by iridescent peridium, well-developed white calcareous stipe, and a conspicuous white, central, calcareous nucleus. It is easily differentiated from *P. nucleatum* Rex which possess noncalcareous stipe, non-iridescent peridium, and a dense capillitium.

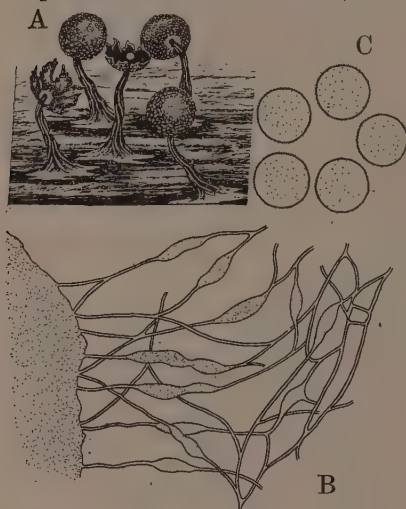
### 53. *Physarum pusillum* (Berk. & Curt.) G. Lister

*Fructifications* sporangiate: sporangia gregarious or scattered, stipitate, erect or sometimes inclined globose, small, white, or ashen white, brown at the base, 0.38 – 0.5 mm. in diameter: stipe long, slender, erect to bent, or curved at the top, gradually tapering upward, dark brown, lighter coloured above, longitudinally rugose, noncalcareous, 0.6 – 1.3 mm. long: hypothallus dark brown, well developed, rotate: peridium membranous, single, covered over with numerous, white, irregular flakes or scales of lime, lime flakes absent on the brown base: dehiscence irregular, usually by longitudinal splitting.

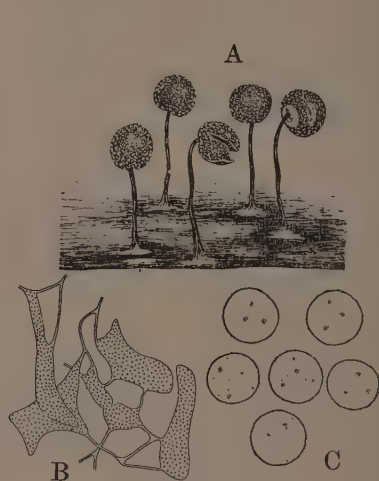
*Columella* absent.

*Capillitium* abundant, uniform and not variable, composed of a network of nodes and internodes: nodes numerous, white, calcareous, fusoid to elongated and irregular, often joined with one another: internodes slender, hyaline, non-calcareous, well-developed, branched threads.

*Spores* black in a mass, bright purple under the microscope, globose, minutely and profusely verrucose, warts also aggregated into large, prominent clusters,  $9 - 10.8 \mu$  in diameter.



Text-Fig. 4



Text-Fig. 5

Text-Fig. 4. *Physarum stellatum* (Masse) G. W. Martin, A. Sporangia,  $\times 20$ . B. Capillitium with small, fusiform nodes,  $\times 400$ . C. Minutely verrucose spores,  $\times 1000$ .

Text-Fig. 5. *Physarum pusillum* (Berk. & Curt.) G. Lister, A. Longstipitate sporangia,  $\times 20$ . B. Capillitium,  $\times 400$ . C. Spores with prominent clusters of warts,  $\times 1000$ .

Collected on dead leaves of *Agave* species, Adunca Bridge Mussoorie, September 8, 1956, 169. New record in India.

This Mussoorie collection undoubtedly belongs to *Physarum pusillum* (Berk. & Curt) G. Lister. It shows close relationship with the European collections and shows the same characteristics as given in Lister's monograph, (1925). It differs from the American collections (described in Martin's Monograph, 1949) in the distribution of warts on the spore wall. The spores in the American collections seem to be devoid of clusters of warts which are so prominently present in the Mussoorie collection and have also been reported in the European collections.

#### 54. *Physarum sulphureum* Alb. & Schw.

*Fructifications* sporangiate: sporangia gregarious, or scattered, stipitate, erect, orange yellow at the top and dull greenish yellow below,

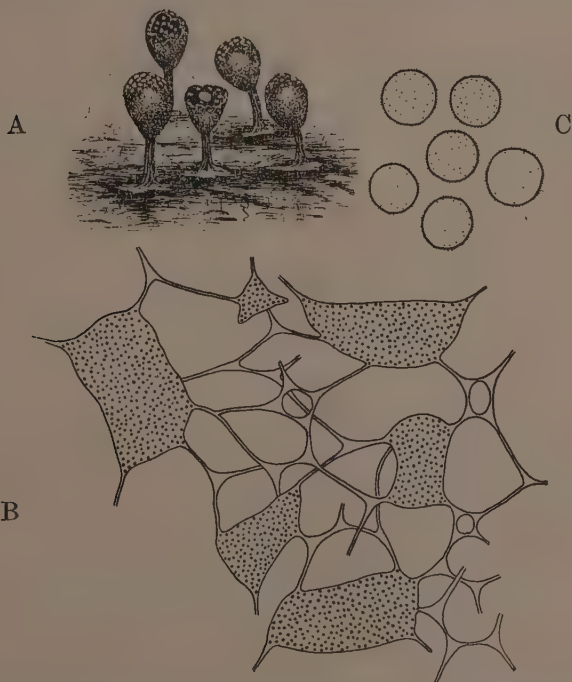
obovate or tubinate (never globose)  $0.38 - 0.5 \times 0.28 - 0.36$  mm. stipe yellowish brown, uniform in width throughout, rugulose, calcareous,  $0.23 - 0.35$  mm. long, i.e. about two-third of the height of sporangia: hypothallus small, dark brown, membranous, rotate: peridium single, thick, crustaceous, opaque, covered at the top with orange yellow calcareous squamulose deposits: dehiscence irregular, the lower portion remaining very persistent like a cup.

*Columella* none.

*Pseudocolumella* present in the form of a conspicuous, large, yellow, calcareous spherical mass in the centre formed by aggregation of the nodes of the capillitium.

*Capillitium* abundant, intricate, composed of nodes and internodes; Nodes large, yellow, some lighter coloured and a very few almost white, angularly elongate, irregular in shape and size, interconnected by delicate, very fine but abundant intricate and noncalcareous, internodes.

*Spores* black in mass, violaceous brown under the microscope, globose, profusely and minutely verrucose,  $8 - 9.6 \mu$  in diameter, mostly  $8 \mu$  in diameter.



Text-Fig. 6. *Physarum sulphureum* Alb. & Schw., A. Turbinate sporangia, with one sporangium showing a pseudocolumella, x. 20. B. Capillitium x 400. C. Minutely verrucose spores, x 1000.



Collected on dead leaves, Chakrata Toll, Mussoorie, September 1, 1955, 170.

This collection undoubtedly belongs to *Physarum sulphureum* Alb. & Schw. and is characterized by turbinate (or obovate) sporangia, yellow nodes, and a yellow pseudocolumella in the centre. The shape is uniformly obovate and not so variable as reported for the species. Its spores are slightly smaller for the species. The yellow nucleate and central pseudocolumella is present in all the sporangia examined.

It is easily differentiated from *Physarum nucleatum* Rex collected and described from the Mussoorie Hills (Thind & Sohi, 1955) which possesses globose, grayish white sporangia, much longer stipes, white nodes and white pseudocolumella.

55. *Lepidoderma tigrinum* (Schrad.) Rost.

*Fructifications* sporangiate: sporangia gregarious, sometimes scattered, stipitate, subglobose, depressed, umbilicate below, erect, 0.55 – 0.85 mm. in diameter: peridium dark gray or dark orange brown, cartilaginous, tough, slightly wrinkled, opaque, covered over superficially and incompletely with numerous circular, crystalline, shining transparent, yellowish orange, flat plates of lime. measuring up to 80  $\mu$  in diameter: stipe 0.35 – 1 mm. long, up to 0.4 mm. wide at the base, stout, slightly tapering upward dull orange coloured, irregularly ridged, ridges small calcareous, solid: hypothallus prominent, common to several adjoining sporangia, or discrete, dull orange or concolorous with the stipe: dehiscence irregular.

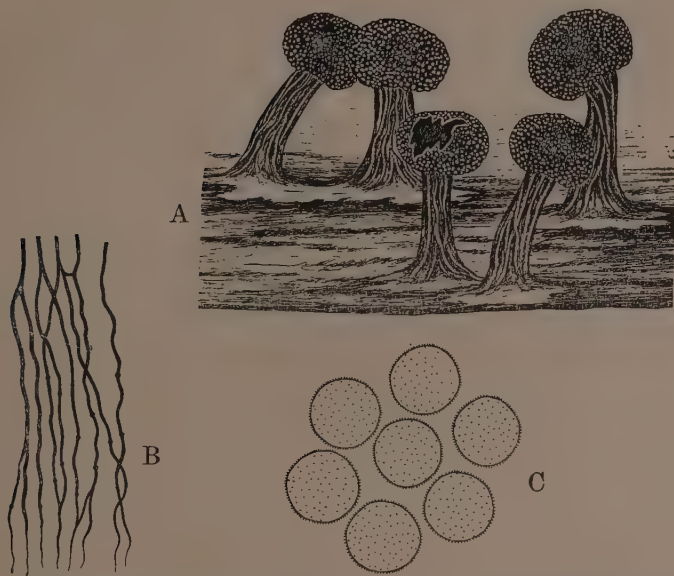
*Columella* well developed hemispherical, orange, i.e. concolorous with the stipe, calcareous.

*Capillitium* abundant, violaceous brown, composed of slender, non-calcarious, straight to flexuous, sparsely branching and sparsely anastomosing threads, hyaline at the extremities, arising from the columella, marked by numerous, darker coloured nodular thickenings.

*Spores* black in a mass, dark violet brown under the microscope, globose, minutely and profusely verrucose, 10–12  $\mu$  in diameter.

Collected on dead wood, Kodia Forest, Mussoorie September 12, 1955, 171. New record in India.

This fungus undoubtedly belongs to *Lepidoderma tigrinum* (Schrad) Rost. and is easily recognized by the stipitate sporangia beautifully covered over with numerous circular calcareous, placoid plates.



Text-Fig. 7. *Lepidoderma tigrinum* (Schröd.) Rost., A. Sporangia beautifully covered over with placoid, calcarious plates,  $\times 20$ . B. Capillitial threads marked by numerous nodular thickenings,  $\times 400$ . C. Minutely verrucose spores,  $\times 1000$ .

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## A NEW RECORD OF *PIRICULARIA* FROM INDIA

P. GOVINDA RAO AND D. KOTESWARA RAO.

(Accepted for publication October 10, 1957)

The genus *Piricularia* Sacc. causes leaf spots on a number of host plants belonging mainly to Gramineae. It includes a few species of which *P. oryzae* Cav. is of great economic importance inciting blast disease of rice throughout the world. The same genus occurs in India on a few grass weeds, namely *Panicum repens*, *Digitaria marginata*, *Dinebra retroflexa* and *Leersia hexandra*. Recently *P. oryzae* var. *commelinae* has been reported on *Commelina benghalensis* from Poona (Thirumalachar et. al 1956). Species of *Piricularia* allied to *P. oryzae* were reported from Malaya (Anonymous 1951) on *Brachiaria mutica* and *Oryza minuta* inciting leaf spots. But the report does not contain their pathogenicity tests and species delimitation. A brief account of morphology, pathogenicity tests and identity of *Piricularia* occurring in India on *Brachiaria mutica* Stapf a graminaceous plant, is furnished below.

*Brachiaria mutica* Stapf. which is a native of America and West Africa is an excellent fodder grass. It is now grown commonly as a perennial fodder grass under wet land conditions in South India. During November 1951, a leaf spot disease was observed on *Brachiaria mutica* at the Agricultural College Farm, Bapatla (Andhra Pradesh). Since then the disease has been prevalent every year. The infection spots were sunken, straw coloured surrounded by a narrow, dark brown margin and measured. 2–7 mm. long and upto 2.5 mm. broad. In moist weather the lower surface of the lesion, was covered with dense, velvet olive green growth of the fungus bearing conidia and conidiophores. The spots may coalesce causing crinkling and drying of the leaf blade.

Microscopic examination of the sections of the lesion revealed hyaline, septate hyphae which measure  $1.5\ \mu$  thick. These emerged out of the epidermis and formed numerous conidiophores. These were slightly dark at the bottom and hyaline at the top, slightly tapering, often in clusters, swollen at the base, 2 to 4 septate, unbranched and geniculate, measuring  $69-106 \times 3-4.6\ \mu$ . The conidia were obclavate to pyriform, hyaline, 2 septate with a small persistent stalk at the base. They measured on average  $\mu\ 17-31 \times 7.7-9\mu$ .

The fungus was brought into pure culture by the transfer of a single germinating spore. The culture grew well on oat meal agar medium with grey aerial mycelium. Sporulation was fairly good in the first subcultures and gradually decreased in later subcultures. Pathogenicity tests were carried out with the isolate by inoculating *Brachiaria mutica*, rice (CO. 13, susceptible to blast) *Eleusine coracana*, *Setaria italica* and *Pennisetum typhoides*. Spore suspension was sprayed on the seedlings grown in pots.

Fresh foliage of *Brachiaria mutica* was obtained by planting cuttings of the grass in pots. The inoculated seedlings were immediately incubated in moist chambers. The experiments were carried out in that period of the year when there was plenty of disease in the field on all the hosts tried except *P. typhoides*. The details of the inoculation experiments are given below,

Host.	Number inoculated.	Number infected.	Interval in days	Results of infection
1. <i>Brachiaria mutica</i>	15	11	4	brown, oval spots measuring 5—6 mm. x 2 mm. developed with olive green growth on both sides.
2. Rice CO. 13 (susceptible to blast)	20	Nil	—	
3. <i>Eleusine coracana</i>	11	Nil	—	
4. <i>Setaria italica</i>	38	Nil	—	
5. <i>Pennisetum typhoides</i> .	9	Nil		

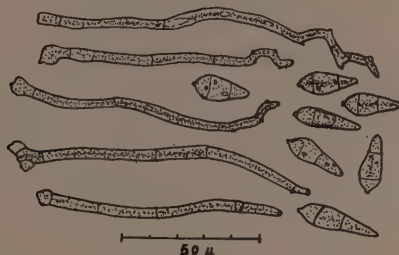
Suitable controls were maintained and these remained healthy throughout the period of experiment. The isolate was readily pathogenic only to its own host forming typical lesions and did not infect at all any other host tried. Similar results were obtained even when the experiment was repeated.

IDENTITY OF THE ISOLATE: The isolate resembles *P. oryzae* Cav. in its general morphology and foliar symptoms. Regarding the size of the conidia of *P. oryzae* Cav. considerable variation is revealed by a glance at the following data recorded by different investigations.

Authority.	Size of the conidia in $\mu$	
	Length	Breadth.
Cavara (Padwick, G.W. 1950)	20—22	10—12
Nishikado (Padwick, G. W. 1950)	14—40	6—13
Usual size	19—23	7— 9
Ramakrishnan K. V. (1948)	21—37	11—15
Average	29	13

Thus it is seen that the size of the conidia of *P. oryzae* varies from 14–40  $\mu$  in length and 6–15  $\mu$  in breadth and the conidia of the present isolate which measure 17–31  $\times$  7.7–9  $\mu$  fall within these ranges, showing its closer affinity with *P. oryzae*. However, repeated inoculation experiments proved that the present isolate infects no other host except its own. To indicate this specialised pathogenicity it is proposed to designate the isolate under study as *P. oryzae* Cav. f. *brachiariae* forma nov. Type specimens of the disease were deposited in Herb. Crypt. Ind. Orient., Indian Agricultural Research Institute, New Delhi.

TEXT FIGURE.



Conidiophores and Conidia.

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Agricultural College, Bapatla,

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# ALTERNARIA LEAF SPOT OF *RICINUS COMMUNIS* L.

V. H. PAWAR AND M. K. PATEL

(Accepted for publication October 15, 1957)

Reports of Alternaria leaf spot on *Ricinus communis* L. in India were made by Dastur (1913), Chibber (1914), Dey (1945) and Singh (1955). Singh (1955) confined his studies mainly to the morphological characters, and, since the disease has been assuming serious proportion in Bombay State, studies were undertaken on its physiological characters and specially the host range.

The first appearance of the disease is observed on the cotyledons which become spotted, remain stunted and die when the infection is extensive. Leaf spot is observed throughout the year but more so in the rainy season when premature defoliation also occurs. The spots which vary from 2 - 14 mm. in diameter are irregular, scattered, with concentric rings and form big patches when coalescent. The affected portion is first yellow turning light brown later. Green capsule, if affected, develops poorly with under developed light seeds. Inflorescence at any stage of development is susceptible to infection.

**MORPHOLOGICAL CHARACTERS.** The mycelium in young cultures on potato dextrose agar is fluffy and spreading. The young hyphae are hyaline, septate, and branched irregularly. As the hyphae get older, the colony colour changes from white to deep olive gray.

The measurement of about 200 conidia produced by the fungus on various media are given below:—

Medium.	Conidia with beak.		Beak.	
	Range microns.	Average microns.	Range microns.	Average microns.
Host decoction agar + Dextrose.      x	81.9-208.1 18.9-31.4	161.01x25.5	33.9-157.71	98.7
Host decoction agar.	83-214.2x 19.32-38	153.08x32.97	32.55-157	91.88
Richard's agar.	86-210 x 20.91-39.64	163.03x34.23	37.93-193.37	99.65
Host lesion.	61.23-245 x 17.29-39.81	168.47x27.93	59.91-195.7	102.87

These measurments compare favourably with those given by Stevenson (1944).

On the host, under moist conditions, the conidia are produced abundantly on affected parts. The conidiophores measure 30–60 $\mu$  in length and each bears conidia in chains at the apex. In most cases, the conidiophores are straight, erect or irregularly bent. The colour of the conidia is dark brown. In culture, the conidia measure 49.35–85.50  $\times$  18.19–38.0 microns exclusive of beak and the beak is 32.55–195.70 microns in length. The number of main cross walls varies from 4 to 14. The conidia are sometimes obclavate, rarely obovate, having rounded bases and tapering gradually to the apex which may be drawn into a nonseptate beak.

**PHYSIOLOGICAL STUDIES.** The best growth was obtained in oat meal agar, moderate in limabean agar, Richard's agar, potato dextrose agar, Brown's medium, host decoction agar with and without dextrose, poor in plain agar, as expressed by colony diameter and density of mycelial mat. The central portion of the colony was raised in case of oat meal agar and Richard's agar. Colony was convex in case of limabean and potato dextrose agar. Sporulation was profuse in Richard's agar and host decoction agar with and without dextrose. In Brown's medium and plain agar, it was scanty while in limabean, oat meal and potato dextrose agar it was totally absent.

The results showed that the fungus grew in temperature range of 7°–38°C., with optimum at 27°–29°C. Low temperature seems to favour sporulation.

Using modified Richard's medium without potassium nitrate as a basal medium with 1.0% inorganic nitrogen and 0.1% organic nitrogen and incubated at 23°C. for 8 days, it was found that urea is the best source of nitrogen for fungus growth. Good growth was obtained in asparagine, aspartic acid and ammonium tartarate, moderate in ammonium sulphate, sodium nitrate, potassium nitrate, arginine, creatine, methionine, guanidine hydrochloride, norvaline, proteose peptone, alanine, norleucine, peptone, glutamic acid, poor in ammonium phosphate, ammonium nitrate, tyrosine, tryptophane, glycocyamine, glycine and modified Richard's agar without potassium nitrate, while in sodium nitrite growth was completely inhibited. Sporulation was profuse in creatine, glycocyamine, peptone, aspartic acid, glycine and glutamic acid; good in asparagine, norvaline, proteose peptone, fair in ammonium tartarate, ammonium phosphate, ammonium nitrate, ammonium sulphate, sodium nitrate, arginine, methionine, tryptophane and Richard's agar without potassium nitrate, and scanty in tyrosine, guanidine hydrochloride, urea, alanine and norleucine.

Using Richard's medium without sucrose with 1.0% carbon compounds and incubated at 23°C., for 8 days, it was found that the fungus can utilise several carbon compounds for its growth. It formed best growth in xylose, maltose, raffinose, levulose, dextrin, inulin and starch, moderate in galactose, lactose, salicin and glycerol, poor in sucrose, dulcitol, arabinose and mannitol. Scanty spores were found in maltose, dextrose, inulin and sucrose while in starch, galactose, raffinose, lactose, xylose, dulcitol, dextrin, salicin, arabinose, glycerol, mannitol and levulose they were absent. From the results, it is clear that the carbon compounds do not help in sporulation of the fungus.

The results show that the fungus can grow in a wide range of hydrogen-ion concentrations, growth decreasing with increase in acidity and alkalinity. The range of optimum reaction lies between 5 and 6 and in general, the amount of aerial mycelium produced in culture is greater in acid than in alkaline reaction. This indicates that acid conditions are more favourable for growth than alkaline conditions.

**GERMINATION STUDIES.** In order to find the time required for germination of spores, hanging drop cultures were made in sterile water. The spores were taken from 8-10 days' old culture on host decoction-dextrose agar and incubated at 26° - 28°C. Germination started after 2 hours. Germ tubes enlarged slightly after 3 hours but at the end of 4½ hours, were large enough to be quite distinct. Nearly 78% of the spores were found germinated. Under these conditions, therefore, the time limit was fixed at 5 hours for germination. The results are given below.

Temperature in °C	0	5	10	22	27	30	35	40
Percent germination.	0	Trace	36.5	61.9	90.7	81.3	43.1	5.8

**HOST RANGE.** In order to test the ability of the fungus to infect hosts other than castor, the following plants were used: *Gerbera jamesonii* Bolus, *Solanum melongena* L., *Euphorbia geniculata* Ortega, *Euphorbia hirta* L., *Euphorbia thymifolia* L., *Acalypha indica* L., *Euphorbia pulcherrima* Willd., *Gossypium* sp., *Bridelia hamiltoniana* Wall, *Jatropha pandurifolia* Andr., and *Ricinus Communis* L. The plants were kept in a moist chamber before and after inoculation. The experiment was repeated three times. The fungus in addition to *Ricinus communis* also infects and incites diseased spots on *Jatropha pandurifolia* and *Bridelia hamiltoniana*.

**IDENTITY OF PATHOGEN.** Fungi like *Macrosporium compactum*, Cke., *M. cavarae* Parisi, *M. nigricans* Atk., *M. ricini*, *Alternaria tenuis* Nees, *A. compacta* (Cke). McClellan, *A. brassicae* (Berk.) Sacc. and *A. ricini* (Yoshii) Hansford have been described to incite leaf spot, stem infection and seedling disease of castor oil plant. From Texas, Cook (1880) described *M. compactum* on mature stem. Dastur (1913) and Chibber (1914) observed an *Alternaria* leaf spot in India. Parisi (1921) described *M. cavarae* on castor plant to cause leaf spot and seedling disease in Italy. Tropova (1928) reported, *M. cavarae* on the cotyledons, leaves, and recemes from Russia. However, Yoshii (1929) described a leaf spot in Korea and Japan and demonstrated by inoculation experiments that the causal organism was a species of *Macrosporium*, which he named *M. ricini*. Bitancourt (1940) recorded species of *Alternaria* on castor plants causing leaf spot in Brazil. Weiss (1942) reported species of *Alternaria* associated with castor leaf spot in Florida, Louisiana, Texas and New York. McClellan (1944) described *A. compacta* attacking castor plants in green house. Stevenson (1944) compared the differentiating characters of *Alternaria* isolated at Beltsville, Maryland (U.S.A.) with other species of *Macrosporium* and *Alternaria* already reported and agreed with Hansford (1943) who changed the designation of *M. ricini* to *A. ricini*. He further believed that with the extreme variability within any one species of the genus *Alternaria*, it is conceivable that the same fungus might have been the cause of the disease

in several and perhaps all of the instances reported in the literature. The following table indicates that the fungus under investigation agrees completely in all respect of spore measurement and symptoms with Stevenson's culture, viz., *Alternaria ricini*, and fairly with that reported by Singh (1955).

Comparison of different species of *Alternaria* attacking castor oil plant.

Authority	Organism	Measurements of spore in microns		
		Width	Length	Beak.
Cook (1880)	<i>Macrosporium compactum</i> Cke.	12-14	20-30	—
McClellan (1944)	<i>Macrosporium compactum</i> Cke.	7-19.5	14-38.5	—
Parisi (1921)	<i>Macrosporium cavaræ</i> Parisi	10-13	34-40	—
Yoshii (1929)	<i>Macrosporium ricini</i> Yoshii	8.7-19.5	42-78	10-30
Stevenson (1944)	<i>Alternaria ricini</i> (Yoshii) Hansford.	15-29	47-96 (Exclusive of beak)	51-200
Singh (1955)	<i>Alternaria ricini</i> (Yoshii) Hansford.	12.6-33.6	29.4-92.4	42-100
Present work.	—	18.9-38.0	49.45-85.5 (exclusive of beak)	32.55-195.7

#### SUMMARY

Castor (*Ricinus communis* L.) suffers from *Alternaria* disease on Poona Agricultural College Farm. Typical concentric spots are produced on leaves. The symptoms of the disease on different parts of the castor are described. The culture produces spores at 20°C.

Morphology and cultural characters of the fungus have been described.

The minimum temperature for growth lies between 5° and 10°C, optimum near 28°C., and the maximum between 35°C and 40°C. The optimum temperature for the germination of spores lies between 25° and 30°C.

The fungus requires organic sources of nitrogen like asparagine, asparatic acid and carbon compounds like xylose, maltose, raffinose etc. and grows best between pH 4.8 and 5.5.



The fungus attacks *Jatropha pandurifolia*, and *Bridelia hamiltoniana* in addition to its host *Ricinus communis*. The morphological, cultural and physiological characters of the fungus align it with *Alternaria ricini* (Yoshii) Hansford from which it is indistinguishable

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## PHOMOPSIS BLIGHT AND FRUIT-ROT OF BRINJAL

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**INTRODUCTION.** A severe leaf blight and fruit-rot disease of brinjal (*Solanum melongena* L.) was noticed at Ugar, Belgaum district, during the winter of 1952. The symptoms of the disease closely resembled those of Phomopsis-rot described by Halsted (1892), Harter (1914) and Nolla (1929). The disease, though previously recorded in Bombay State, had never assumed such an epiphytotic condition and hence it was considered desirable to make a detailed study of the causal organism. The results of these investigations are given here.

**SYMPTOMS.** The disease symptoms range from seedling blight to that of fruit rot. "Damping-off" of seedlings results from infection of the stem just above the soil surface. The disease is, however, more prominent on the leaves during the early stage when it manifests itself in the form of spots, which in the beginning are small, more or less circular, buffy olive (R.) and later turn to cinnamon-buff (R.) with irregular blackish margins. The old leaf spots are irregular in shape and vary from 3 to 16 mm. in diameter. Lesions at a petiole or at a lower part of midrib cause the death of the entire leaf. The blight symptoms are generally prominent under humid conditions. Affected leaves drop prematurely, and the infection spot is covered with numerous pycnidia. On the stem, the disease manifests itself in the form of elongated blackish brown lesions. The leaves on the diseased plants are usually smaller in size, and axillary buds are often killed. When there is girdling of the stem due to infection, the shoot above wilts and dries up. On the fruits, the disease appears first as minute, sunken, dull and dusky purple spots which later merge forming large rotten areas. Once established on the fruit, the disease continues to develop until the entire fruit rots. On such fruits, numerous pycnidia develop.

**MORPHOLOGY.** The fungus produces profuse mycelial growth on Richard's, limabean and potato-dextrose agars, when incubated at 22-24°C. The mycelium consists of fine, hyaline, septate hyphae, which measure 2.7 - 3.9  $\mu$ . in diameter. The pycnidia are minute, black, subepidermal, erumpent and ostiolate. Mature pycnidia are flask-shaped, measuring 80-360  $\mu$  in diameter and the beaks measure 22.2-93.1  $\mu$ . in length. The pycnidiospores are of one type, numerous, and extruded out of the ostiole cirrhi. The pycnidiospores are one-celled, elliptical and biguttulate. This is the common type of spore developing in nature and also on artificial media. On diseased fruits, stem and leaves, spores measure 4.0 - 6.8 x 2.3 - 2.7  $\mu$ . Almost the same measurements are encountered on various agar media.

**CULTURAL CHARACTERS.** The best growth was obtained on limabean, potato dextrose, host decoction dextrose and oatmeal agars as expressed by the colony diameter and density of the mycelial mat. The mycelial growth was fair on Brown's and Richard's agars, while it was very poor on host decoction and plain agars. The pycnidial formation was abundant in host decoction agar with and without dextrose; moderate in Richard's agar; poor in limabean, plain, Brown's and oatmeal, and nil on potato-dextrose agar.

**PHYSIOLOGICAL CHARACTERS:** The fungus has a wide range of growth temperature, the optimum being 28°C. and minimum and maximum at about 9° and 40°C. respectively. The pycnidial formation was abundant between 30° and 35°C; scanty between 11° and 28°C. and none at 5°C. and below. The optimum temperature for germination of conidia is 27°C. This is in accord with the general observations made in respect of the development of the disease in nature and the behaviour of the fungus in culture. Germination generally starts after four hours.

In Richard's modified medium with several carbon compounds added in one per cent concentration, a wide variation in growth rate was observed with different sources of carbon. The fungus grew profusely in media containing dextrose, amygdalin, dextrin, levulose, maltose and sucrose; moderately on raffinose, galactose, glycerin, inulin, xylose and mannitol whereas it made poor growth on starch, salicin, arabinose and lactose. Pycnidial formation was the best on media containing lactose, salicin, arabinose and starch; moderate in sucrose and raffinose and scanty or nil in galactose, dextrose, amygdalin, glycerin, inulin, dextrin, levulose, xylose, maltose and mannitol.

Sucrose seems to be the best source of carbon as compared to others for mycelial growth and formation of pycnidia. (Table I).

The fungus is found to derive its nitrogen requirements from a variety of organic and inorganic sources. The best growth was obtained on proteose peptone, peptone, sodium nitrate and potassium nitrate; moderate on ammonium tartarate, glycine, methionine, asparagine, glyco-cyamine, tyrosine, creatine, guanidine hydrochloride, arginine, alanine, aspartic acid and urea; poor on norvaline, norleucine, tryptophane, glutamic acid, ammonium nitrate, ammonium sulphate, whilst sodium nitrite was toxic. In the absence of nitrogen, the fungus makes poor growth. The type of growth, topography and colour of the colonies also vary greatly with different nitrogen sources. Zonation was produced on media containing proteose peptone, glycine, methionine, norleucine, guanidine hydrochloride, arginine, alanine, aspartic acid, urea, sodium nitrate and ammonium tartarate. The production of pycnidia is either absent or scanty in inorganic nitrogen. The best formation of pycnidia is on asparagine, glutamic acid, arginine and alanine; moderate on glycine, tyrosine, peptone, norvaline, guanidine hydrochloride, aspartic acid and urea; poor on tryptophane, norleucine and methionine, and absent on proteose peptone, glyco-cyamine and creatine. (TABLE II).

The fungus grows in a wide range of hydrogen-ion concentration, namely 2.6 to 9.9 with optimum reaction lying between 4.4 and 5.8. The amount of aerial mycelium produced in culture is greater in acid than in alkaline reaction. However, the pH of the filtrate tends towards neutrality.

TABLE I. Growth of brinjal *Phomopsis* on Richard's agar containing different carbon compounds.

Source of Carbon	Colony diameter in mm.	Pycnidial formation.	Growth characters of mycelium.
Amygdalin.	65	0	Aerial, pale vinaceous fawn.*
Arabinose.	47	+++	Submerged, hyaline.
Dextrin.	62	+	Aerial, vinaceous fawn.
Dextrose.	65	+	Aerial, light drab.
Galactose.	55	+	do
Glycerine.	57	0	Aerial, pale vinaceous buff.
Inulin.	57	0	Aerial, hyaline.
Lactose.	52	+++	Submerged, hyaline.
Levulose.	60	0	Aerial, vinaceous buff.
Maltose.	60	0	Aerial, vinaceous fawn.
Mannitol.	55	0	Aerial, vinaceous buff.
Raffinose.	50	++	Aerial, light drab.
Salicin.	38	+++	Submerged, hyaline.
Starch.	57	+++	Submerged, pale vinaceous fawn.
Xylose.	55	0	Aerial, vinaceous fawn.
Control (Richard's agar with 5% sucrose).	61	++	Aerial, light drab.
Control (Richard's agar with 1% sucrose).	60	++	do

+++ Abundant, ++ Moderate, + Scanty and 0 Nil.

\*Colour according to Ridgway's colour standards.



TABLE II. Growth of brinjal *Phomopsis* on modified Richard's agar containing different nitrogen sources.

Source of nitrogen.	Colony diameter in mm.	Pycnidial formation.	Growth characters of mycelium.
Alanine	55	+++	Aerial, light olive gray.
Arginine.	54	+++	Aerial, olive gray.
Asparagine.	52	+++	Aerial, light drab.
Aspartic acid.	59	++	Aerial, hyaline.
Creatine.	59	0	Partly aerial, pale vinaceous pink.
Glutamic acid.	48	+++	Aerial, light olive gray.
Glycine.	54	+	Aerial, light drab.
Glycocyamine.	57	0	Subaerial, olive gray.
Guanidine hydrochloride.	52	++	Aerial, flesh pink.
Methionine.	52	+	Aerial, light olive gray.
Norleucine.	50	+	Aerial, deep olive gray.
Norvaline.	41	++	Aerial, deep olive gray.
Peptone.	67	++	Aerial, light olive gray.
Porteose peptone.	70	0	Aerial, deep olive gray.
Tryptophane.	46	+	Aerial, deep olive gray.
Tyrosine.	59	++	Aerial, deep olive gray.
Urea.	57	++	Aerial, light drab.
Control (Modified Richard's agar)	49	++	Subaerial, tea green.
Ammonium nitrate.	40	0	Aerial, tiller buff.
„ sulphate.	39	0	Aerial, pale vinaceous fawn.
„ tartarate.	54	0	Aerial, light vinaceous pink.
Potassium nitrate.	61	+	Aerial, vinaceous buff.
Sodium nitrate.	64	0	Aerial, vinaceous buff.
„ nitrite.	0	0	No growth.

Please see explanation under Table I.

VARIETAL RESISTANCE AND HOST RANGE: Of the 24 varieties of brinjal tested, none was found to be resistant. The fungus infects *Solanum melongena* only but not *Capsicum annuum* L., *Datura fastuosa* L., *Lycopersicum esculentum* Mill., *Nicotiana tabacum* L., *Solanum nigrum* L., *Solanum tuberosum* L. and *Petunia* sp.

\*TAXONOMY: Spegazzini seems to be the first to describe the disease in 1881. Halsted (1892) was the first to study it in detail. Rolfs reported it from Florida in 1933. Subsequently, it has been reported from other States. Nolla (1929) considers the disease to be endemic as it was found on new lands where vegetables had never grown before. According to him, a number of identical diseases have been described on egg plant and it is but natural that various names were given to the causal agent. Spegazzini (1881) described *Phyllosticta hortorum* Speg. on the egg plant leaves and fruit. Halsted (1892) found it in New Jersey on brinjal leaves and fruit. He attributed the damping-off or seedling stem-blight to *Phoma solani* Hals. However, Nolla (1929) could show that *P. solani* like *Phyllosticta hortorum* was able to produce some lesions on all the above-ground parts and in plants of all ages and he, therefore, felt that only one name should hold. Smith (1904) reported *Ascochyta lycopersici* Brun. causing seedling blight in egg plants and considered it to be different from *P. hortorum* as he found differences in size and septation of the spores and in the symptoms on the leaves. In a later paper, Smith (1904) regarded *A. lycopersici* and *P. hortorum* to be identical since both produced seedling blight in egg plants. Harter (1914) concluded that the name *A. hortorum* (Speg.) Smith should hold, for the reasons of priority. However, after examination and study of material from New York, Nebraska, New Jersey and Wisconsin he (1914) found that the fungus in question was characteristically a member of the genus *Phomopsis* and concluded like Halsted that this fungus and *P. solani* were identical. Saccardo and Sydow (1899) named *Phoma vexans* to Halsted's fungus since *P. solani* had already been applied to another species. Harter (1914) then suggested a new combination *Phomopsis vexans* (Sacc. and Syd.) Harter. Harter sent material to Spegazzini for comparison with type specimens of *P. hortorum* and the latter apparently found them to be different on the basis of pycnidiospore and pycnidial measurements. From host relationships, morphological characters and spore measurements, Harter (1914) was of opinion that Smith had both, *Ascochyta* and *Phyllosticta* on the same host.

In the table below, spore and pycnidial measurements for *P. hortorum*, *Phomopsis vexans* and the fungus under study are given for comparison:—

Name of the organism.	Measurements in $\mu$ of		Origin of Pycnidia
	Pycnidiospores Length	Pycnidia Width.	
<i>Phyllosticta hortorum</i>	4-6	2-2.5	80-90 Leaves.
<i>Phomopsis vexans</i>	5-8	2-2.8	60-200 Leaves and stem 120-350 Fruits.
Culture under study.	4-6.8	2.3-2.7	80-220 Leaves and stem 125-360 Fruits.

Comparative studies of the measurements of the pycnidia and pycnidiospores of the fungus under study and *Phomopsis vexans* (Sacc. and Syd.) Harter, from the table above indicate that they are identical. One interesting feature, however, is that the stylospores are very sparsely produced

in the present fungus. The ascigerous stage *Diaporthe vexans* Gratz reported from the United States has not been observed in the material under study.

#### SUMMARY

Brinjal (*Solanum melongena* L.) suffers from *Phomopsis* disease in Belgaum district of Bombay State. Typical flask-shaped pycnidia are commonly produced on leaves, stem and fruit on the affected parts. The symptoms of the disease on the different parts are described. The culture produced elliptical spores under natural conditions and in artificial culture but never produced stylospores.

The morphology and cultural characters of the fungus have been described. Measurements of spores and pycnidia are also given.

The minimum temperature for growth lies between 7° and 11°C., the optimum near 28°C. and the maximum between 35° and 40°C.

The fungus does not attack any of the host plants tried except brinjal. The morphological, cultural and physiological characters of the fungus align it with *Phomopsis vexans* (Sacc. and Syd.) Harter from which it is indistinguishable.

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## EFFECT OF CARBON NUTRITION ON GROWTH AND SPORULATION OF SOME ANTHRACNOSE FUNGI

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The investigations of earlier workers have established that species of fungi vary in their ability to utilize different carbon compounds for growth and sporulation. Tochinnai (1926) found that glucose and sucrose were poorly utilized by *Colletotrichum lini*. Tandon & Grewal (1954) found arabinose, rhamnose & xylose as poor and glycerine, erythritol, sorbitol and mannitol as good sources of carbon for the growth of *Alternaria tenuis*. The studies of Lilly and Barnett (1953) on the rate and amount of growth of 57 fungi upon 12 sugars have clearly established that species of fungi differ in their ability to utilize different sugars.

Hawker (1939) and Westergaard & Mitchell (1947) found that the production of conidia and perithecia of *Melanospora destruens* and *Neurospora crassa* were influenced by the kind and concentration of the sugar used. Kendrick & Walker (1948), Mathur *et al.* (1950) as well as Tandon and Grewal (1954) working on *Colletotrichum phomoides*, *C. lindemuthianum* and *Alternaria tenuis* respectively reported that the source of carbon in the basal medium influenced sporulation to a great extent. It was, therefore, thought necessary to study the growth and sporulation of the three pathogenic fungi on different carbon compounds.

**MATERIALS & METHODS:** The investigations were carried out with *Gloeosporium musarum* Cke. & Mass. isolated from banana, and *Gloeosporium papayae* P. Henn. and *Colletotrichum papayae* P. Henn. from papaya. These fungi were isolated by single spore culture method. On the basis of their growth and sporulation on four different media (viz. Asthana & Hawker's medium A, Coon's synthetic medium, Modified Czapek's medium and Richard's medium); modified Czapek's medium containing.  $\text{NaNO}_3$ ; 2.0 gms,  $\text{K}_2\text{HPO}_4$ ; 1.0 gm,  $\text{KCl}$ ; 0.5 gm.  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ; 0.5 gm, Sucrose; 15 gms,  $\text{FeSO}_4$ ; 0.01 gm and distilled water 1000 c.c. was selected as basal medium. Sucrose of the basal medium was replaced singly by different carbon compounds. Care was taken to provide 6.32 gms of carbon per litre in each case, similar to the amount of carbon in 15 gms of sucrose used in modified Czapek's medium. As the structural formulae of polysaccharides are not known, they were used equal to the amount of sucrose, present in the basal medium. The pH of all the media was adjusted to 5.

Double distilled water, Pyrex glassware and chemicals of purest grade were used throughout the experiment. Liquid cultures containing 50 c.c. of nutrient solution in 150 c.c. conical flasks were sterilized at 15 lb. pressure for 20 minutes. Four replicates of each treatment were incubated at 24°C for three weeks. The dry weight was determined by the



method described by Grewal (1955). Sucrose of the basal medium was replaced by the following carbon compounds.

#### A: CARBOHYDRATES

Monosaccharides		Disaccharides	Trisaccharides	Polysaccharides
Pentoses ( $C_5H_{10}O_5$ )	Hexoses ( $C_6H_{12}O_6$ )	( $C_{12}H_{22}O_{11}$ )	( $C_{18}H_{32}O_{16}$ )	( $C_6H_{10}O_5$ ) <sub>x</sub>
Arabinose	Mannose	Lactose		Starch
Rhamnose	Glucose	Maltose	Raffinose	Dextrin
Xylose	Galactose	Sucrose		Inulin

#### B: ALCOHOLS

Trihydric ( $C_3H_8O_3$ )	Tetrahydric ( $C_4H_{10}O_4$ )	Hexahydric ( $C_6H_{14}O_6$ )
Glycerine	Erytheritol	Dulcitol Mannitol Sorbitol

#### C: GLUCOSIDE: Arbutin ( $C_{12}H_{16}O_7$ )

OBSERVATIONS: No autolysis of the mycelium was observed in any treatment even at the end of the experimental period. The results of dry weight and sporulation were carefully studied and average results are recorded in table 1.

The results of table 1 indicate that there was no growth of *G. musarum*, *G. papayae* and *C. papayae* in the complete absence of carbon. Significantly good growth of the above fungi was recorded on arabinose, rhamnose, lactose, sucrose, galactose, glucose and erytheritol. It was moderate on maltose and ranged from moderate to good on xylose, mannose, raffinose, glycerine & mannitol. The growth of the three fungi was significantly poor on soluble starch, inulin & arbutin.

The effect of carbon compounds on the sporulation of the three fungi was quite interesting. Good sporulation was recorded on arabinose, rhamnose, xylose, mannose, galactose, lactose, maltose, raffinose & sorbitol. It was poor on mannitol & glycerine and was completely checked when dextrin or inulin was used as source of carbon in the medium. Sporulation of the three fungi ranged from poor to good on sucrose, starch, erytheritol & dulcitol. It was noteworthy that all monosaccharides (pentoses & hexoses) used in present investigations supported good sporulation of the three fungi.

TABLE 1. Average dry weight in milligrams and degree of sporulation of *Gloeosporium musarum*, *Gloeosporium papayae* and *Colletotrichum papayae* on different carbon compounds added to the basal medium at the rate of 6.32 gms. of carbon per litre.

Name of Carbon Compound	Average dry weight and sporulation			
	<i>G. musarum</i>	<i>G. papayae</i>	<i>C. papayae</i>	Setae formation
Arabinose	207.7**	185.8**	177.9**	moderate
Lactose	206.3**	152.6**	144.3**	do
Erythritol	196.1**	165.0*	177.2**	poor
Sucrose	193.1**	175.3*	148.1**	moderate
Rhamnose	188.3**	162.1**	168.9**	do
Mannitol	185.4†	138.8†	152.8†	do
Galactose	182.3**	171.0**	146.2**	do
Xylose	178.8**	159.2**	127.1**	do
Glucose	166.0**	143.4**	139.2**	do
Glycerine	151.5†	138.8†	132.4†	absent
Raffinose	142.1**	158.7**	142.9**	poor
Mannose	140.9**	143.5**	134.3**	moderate
Maltose	125.4**	124.7**	111.1**	poor
Dextrin	112.1†	133.4†	136.3†	absent
Dulcitol	105.5**	167.9*	143.6†	poor
Starch	101.2*	91.3†	85.7*	absent
Arbutin	96.4†	69.0†	77.3†	do
Sorbitol	88.5**	96.0**	167.7**	do
Inulin	5.3†	18.1†	8.9†	do
Control (No Carbon)	0.0†	0.0†	0.0†	do

† indicates sporulation Absent; †sporulation Poor; \* Sporulation Fair; and\*\* Sporulation Good)

Note:- Poor denotes 1-5 spores; fair 6-10 spores & good 16 and more spores per low power of microscopic field.

Summary of results at 1% level of probability.

	Replications	...	...	...	non significant
	Treatments	...	...	...	highly significant
Fungus	General mean	S.E.	C.D. at 1%		
<i>G. musarum</i>	138.6	1.59	5.99		
<i>G. papayae</i>	129.7	3.08	11.63		
<i>C. papayae</i>	126.0	1.89	7.12		

General mean  $\pm$  C.D. at 1% level = moderate growth.

The formation of setae in *Colletotrichum papayae* was also influenced by the nature of the carbon compound added to the basal medium. Their number was poor on maltose, raffinose, erythritol & dulcitol and moderate on all other carbon compounds except on starch, dextrin, inulin, arbutin, glycerine & sorbitol where they were completely absent.

**DISCUSSION AND CONCLUSIONS:** Arabinose and lactose supported good growth of the three fungi used in present investigations. Hawkins (1915) working on *Glomerella cingulata* and Kendrick and Walker (1948) working on *Colletotrichum phomoides* obtained similar results with arabinose and lactose respectively. Leach (1923) obtained good growth of *Colletotrichum lindemuthianum* on raffinose. Similar growth was recorded on it with *G. papayae* and *C. papayae*. Tochinai (1926) found inulin to be poor source of carbon for *Colletotrichum lini*. The three fungi used in present study also gave poor growth on it.

Xylose and lactose supported good sporulation of *G. musarum*, *G. papayae* and *C. papayae*. Mathur *et al.* (1950) as well as Kendrick and Walker (1948) also obtained good sporulation of the fungi investigated by them on xylose and lactose respectively. Kendrick and Walker (1948) reported that setae of *Colletotrichum phomoides* were best developed when starch or dextrin was used as a sole source of carbon. In case of *C. papayae* setae were not formed on them at all.

Monosaccharides in general induced good sporulation and arabinose supported best growth and sporulation of the three fungi used in present investigations. There was very little correlation in the amount of growth and degree of sporulation supported by various carbon compounds.

#### SUMMARY

Effect of 19 carbon compounds on growth and sporulation of *Gloeosporium musarum*, *Gloeosporium papayae* and *Colletotrichum papayae* was studied. There was no growth of the fungi in the absence of carbon. Arabinose, rhamnose, lactose, sucrose, glucose, galactose and erythritol supported good growth of the three fungi. Growth was, however, significantly poor on starch, inulin and arbutin.

All monosaccharides, maltose, lactose, raffinose, and sorbitol supported good sporulation. It was poor on mannitol and glycerine and was completely checked on dextrin and inulin. In case of *C. papayae* presence, absence and number of setae was affected by the nature of carbon compound added to the basal medium.

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## IN VITRO ACTION OF MYCOSTATIN ON FUNGI PATHOGENIC TO BANANA

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Mycostatin, the trade-name for the Squibb preparation of nystatin, also referred to as St. 695 or fungicidin, is an antifungal antibiotic reported to be effective on several fungi pathogenic to man (Hazen and Brown 1950, Raubitschek *et al* 1952, Tarbet *et al* 1953). With a view to evaluate the antibiotic in plant disease control, studies on its effect on plant pathogenic fungi was taken up recently in this laboratory and its action on three fungi, pathogenic to banana, is reported in this paper.

**MATERIAL AND METHODS:** A sample of Mycostatin obtained from Messrs E. R. Squibb & Son, New Brunswick, N. J., U.S.A. was used in all the studies. The antibiotic was first assayed against *Candida tropicalis* (Bonor), Didd. & Lodd, by the agar-streak dilution assay method (Waksman and Reilly 1945) and was found to contain 400 units/mg. Fresh isolates of *Fusarium oxysporum* var. *cubense* (E. F. Sm.) Snyder & Hansen, the causal organism of the panamawilt disease of banana, *Gloeosporium musarum* Cke. & Masee, causing anthracnose of the fruits, and *Helminthosporium torulosum* (Syd.) Ashby, causing leaf, stem and fruit infections of banana, were used in the studies. The effect of Mycostatin on the three fungi was tested by the agar-streak dilution, the spore-germination (Anonymous 1943), and the Culture-disc assay methods (Rangaswami 1956). For the agar-streak dilution and the spore-germination assays, seven day-old sporulating cultures of the fungi, grown on potato dextrose agar slants, were suspended in sterile distilled water and used as the inocula. The inhibition of growth of the fungi in the former case was recorded after 48 hours and the germination counts in the latter case taken after 4, 8, 24, 48, and 72 hours. The inoculum for the culture-disc assay was obtained by growing the fungi in potato dextrose agar plates and cutting 5 mm. discs from the periphery of the colony by means of a cork borer. The plates, on inoculation, were incubated at room temperature (22 to 28°C) and the diameter of the colony recorded periodically.

**EXPERIMENTAL: I. Inhibitory Concentrations:** The experiments on the inhibition of the three fungi by Mycostatin were run simultaneously under identical conditions and the results obtained are summarised in Table I. The concentrations of the antibiotic required for the complete inhibition of germination of the spores were recorded after 48 hours and the LD<sub>50</sub> values obtained by means of semi-logarithmic graphs, taking the germination percentage in the control as the standard. The results from the culture-disc assay were also analysed in a similar manner and the LD<sub>50</sub> values obtained.

TABLE I. The inhibitory concentrations of Mycostatin on three fungi pathogenic to banana : mcg/ml

Fungus	Agar-streak dilution assay	Spore-germination assay		Culture-disc assay	
		Complete inhibition	LD <sub>50</sub>	Complete inhibition	LD <sub>50</sub>
<i>Helminthosporium torulosum</i>	2.5	10.0	7.5	10.0	2.2
<i>Gloeosporium musarum</i>	2.5	12.5	7.5	50.0	10.2
<i>Fusarium oxysporum cubense</i>	10.0	25.0	12.5	1,000	120.0

Mycostatin is comparatively more effective on *H. torulosum* and *G. musarum* than on *F. oxysporum cubense*. In general, the antibiotic seems to be more effective on the spores in agar plates, as found in the agar-streak dilution assay, than in the aqueous solutions of the spore-germination assay.

**II. Effect on Germinating Spores:** Mycostatin completely inhibits the germination of the spores at higher concentrations, but at slightly lower concentrations it seems to interfere with the normal development of the germ tubes, in at least two of the fungi, and at still lower concentrations it has little or no effect on the spores or the germ tubes. The germ tubes of *H. torulosum* and *F. oxysporum cubense* are malformed at 5 and 10 mcg/ml, respectively, in the aqueous solutions (Figs. 1 & 2). The germ tubes are converted into narrowly septate chains of cells, some of which are several times broader than the normal germ tubes. In the case of *G. musarum* the antibiotic causes lysis of the spores at higher concentrations but there is no malformation of the germ tubes at lower concentrations.

**DISCUSSION:** In the present studies the antibiotic has been found to inhibit *in vitro* the three fungi pathogenic to banana, though there were considerable variations in the inhibitory concentrations for the various fungi. The effect of the antibiotic on the mycelium is comparatively lesser than on the spores. This is especially pronounced in the case of *F. oxysporum cubense*; more than 1,000 mcg/ml of the antibiotic is required for the complete inhibition of growth of the mycelium in the culture-disc assay, while in the spore-germination and the agar-streak dilution assays, where mostly the spores are used as the inocula, the inhibitory concentrations are only 25.0 and 10.0 mcg/ml, respectively.

The fungistatic effect of Mycostatin is well expressed in the form of the malformations in the germ tubes. There are some previous records of similar morphological effects on the germ tubes and hyphae of certain fungi caused by a few antibiotics and other metabolic products of micro-

organisms (Porter 1924, Brian *et al* 1946, Nickerson and van Rij 1949, Vasudeva and Chakravarthi 1954). Nickerson and Chung (1954) studying the mechanism of the formation of filamentous hyphae in *Candida albicans* (Robin) Berkh. found that the sulphhydryl group present in the cysteine molecule was responsible for the formation of the budding cells in the yeast. Though it is probable that Mycostatin interferes with the normal metabolism of the germ tube, the mechanism of action of the antibiotic seems to be rather specific, since the molecule does not contain the sulphhydryl group as known at present (Oliver Campen 1956).

#### SUMMARY

The action *in vitro* of Mycostatin on *Helminthosporium torulosum* (Syd.) Ashby, *Gloeosporium musarum* Cke. and Masee, and *Fusarium oxysporum* var. *cubense* (E. F. Sm.) Snyder and Hansen were studied by the agar-streak dilution, the spore-germination, and the culture-disk assay methods. The antibiotic was found to inhibit the growth of the three fungi, but it was relatively more effective on *H. torulosum* and *G. musarum* than on *F. oxysporum cubense*. Malformations of the germ tubes of *H. torulosum* and *G. musarum* were also observed at certain concentrations of the antibiotic in aqueous solutions.

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# RELATIONSHIP BETWEEN ANATOMICAL CHARACTERS OF LEAF AND RESISTANCE TO INFECTION OF *HELMINTHOSPORIUM ORYZAE* IN PADDY

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**INTRODUCTION:** During the course of investigation on the susceptibility of different varieties of paddy to infection of *Helminthosporium oryzae* Breda de Haan., it has been observed that varieties grown under same conditions and exposed to the same degree of infection, vary in their degree of resistance to attack of *Helminthosporium oryzae* (Chattopadhyay and Chakrabarti, 1955). Infection of paddy leaves by *Helminthosporium oryzae* has been observed to take place by direct mechanical penetration of the cuticle (Nisikado & Miyako, 1922). Suzuki (1934) made anatomical studies of the different varieties of rice plant in Japan in relation to their susceptibility to Blast (*Piricularia oryzae* Cav.), and brown spot disease on dry and flooded soils. He observed that the thickness of the outer walls, the thickness of silicated outer most layer of epidermal cells, number of silicated bulliform cells, silicated long and short cells and silicated guard cells of stomata are more in the resistant variety than in the susceptible one. Ikata *et al* (1932) noted that in respect of blast disease, resistant varieties have thicker-walled epidermis than the susceptible ones.

Accordingly, studies were taken up to find out whether variation in resistance noted among the different varieties in this case, is correlated with the characteristic anatomical features of the leaf.

**MATERIALS AND METHODS:** Studies were made on the leaves of five varieties of paddy, namely Patnai 23, Asra 108/1, Bhasamanik, Tilakkatchery and Badsabhog 72. These varieties were chosen as they differ considerably in their degree of susceptibility to infection of *Helminthosporium oryzae*.

The following anatomical characters which have a close relationship with mechanical penetration of the host tissue by the fungus were studied.

(1) thickness of the cuticle, (2) thickness of the epidermal cells, (3) number of silicated epidermal bulliform cells per unit area of observation, (4) number of silicated epidermal long cells present per unit area of observation and (5) percentage of  $\text{SiO}_2$  in the leaf sample.

For the purpose of study, the paddy varieties were grown under identical conditions. From each variety ten samples were taken and twenty observations were made on each sample. Leaves were collected when the plants were about to flower.

Thickness of the cuticle and the epidermal layer was measured from the free hand transverse sections which were stained with 1% safranin.

Number of silicated bulliform cells was estimated by the spodogram method of Warner (1928).

The number of epidermal long cells was determined in the usual way, by taking readings on the scrapings of the epidermal layer.

SiO<sub>2</sub> content was estimated by the gravimetric method (Piper, 1950) and was expressed in terms of percentage of the dry weight of the leaves.

OBSERVATIONS: Observations made on the relationship between leaf infection and certain anatomical characters and SiO<sub>2</sub> content of the leaves in different varieties of paddy are given below.

Variety	Average leaf infection value	Average Thickness of the epidermis (A) in $\mu$	Average Thickness of the cuticle (B) in $\mu$	Total thickness of the layer to be penetrated by the fungus (A+B) in $\mu$	Average number of silicated epidermal long cell	Average number of silicated epidermal bulliform cell	Average Percentage of SiO <sub>2</sub>
Patnai 23	107	5.64	4.06	9.70	128.9	24.2	10.3
Asra 108/1	282	2.59	3.98	6.57	123.9	13.8	23.4
Bhasamanik	105	5.05	3.99	9.04	126.0	24.9	13.3
Tilakkatchery	111	5.21	3.70	8.91	123.6	18.8	22.9
Badsabhog	163	4.24	3.66	7.90	127.5	11.9	13.4

From these results it may be concluded that in the varieties showing higher leaf-infection values, total thickness of the cuticle and the epidermal layer, particularly the latter, is comparatively low. As the fungus often enters into the host by direct penetration, the thickness of the cuticle and the epidermal layer is an important factor in determining resistance. The result, in general, conform to that obtained by Akai and Asada (1954) in respect of three different varieties of paddy in relation to susceptibility to *Helminthosporium oryzae*. There is however no direct proportionality between the leaf infection and the thickness of the cuticle.

Varieties Asra and Basabhog 72 showing comparatively high leaf infection value have fewer number of silicated epidermal, bulliform cells. Adyanthya and Rangaswami (1957, in respect of blast disease of paddy, noted that in susceptible varieties there is comparatively less number of silicated epidermal bulliform cells. Akhi and Asada (1954) also noted fewer number of bulliform cells in the susceptible variety "Magatama" SiO<sub>2</sub> content is variable in different varieties, but this does not appear to have any relationship with the resistance to infection of *Helminthosporium oryzae*.

## SUMMARY

Data were taken of the thickness of the cuticle and epidermis, number of silicated epidermal long cells and epidermal bulliform cells and  $\text{SiO}_2$  content of five different varieties of paddy, showing different degrees of infection, namely, Patnai 23, Asra 108/1, Bhasamanik, Tilakkatchery and Badsabhog. 72.

Varieties Asra 108/1, and Badsabhog 72 with epidermal layer comparatively thinner showed more leaf infection.

Silicated epidermal bulliform cells were less in varieties Asra 108/a, and Badsabhog 72 which were more susceptible to infection of *Helminthosporium oryzae*.

No correlation was observed between  $\text{SiO}_2$  content of the leaf and resistance to infection.

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## FURTHER STUDIES ON THE CONTROL OF LOOSE SMUT OF WHEAT IN THE PUNJAB

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Loose smut of wheat is characterised by the total transformation of ears into a black powder, consisting of myriads of spores of the causal fungus, *Ustilago tritici* (Pers.) Rost. It occurs all over the Punjab State and is particularly serious in the relatively more humid places, like Gurdaspur, Kangra, Hoshiarpur, Jullundur, Ambala and Karnal. Humid weather at blossoming time favours infection to a great extent, and the resulting produce, when used as seed next sowing season, produces a crop with heavy loose smut infection. It has been estimated that the total loss caused by this disease every year in the Punjab State is not less than 2 crores of rupees. A very simple method, totally effective in eliminating the disease, but applicable only in the plains, was discovered in 1929 by Professor J. C. Luthra of the Punjab Agricultural College, and Research Institute, Lyallpur (now in Pakistan). This method is known as solar heat treatment.

Luthra (1933, 1941, 1953,) recommends that for the solar treatment to be effective, the seed wheat, soaked for 4 hours from 8 A.M. to 12 noon, should be exposed to the sun till 4 P.M. Also there has been existing a notion among the members of the Agriculture Department of the Punjab State that for the effective elimination of loose smut infection from soaked seed wheat, it has necessarily to remain under the influence of solar rays for a number of hours, not less than four. It is thought that, if the weather during the period of exposure to the sun becomes windy or cloudy, the treatment remains incomplete. To ascertain whether these fears had a basis or were only groundless, regular experiments have been carried out during the summer months of the years 1952, 1953 and 1954 in the Section of Plant Pathology, Government Agricultural College and Research Institute, Ludhiana.

For these experiments, naturally-infected seed wheat was taken and was soaked in ordinary water at room temperature for 4 hours from 8 A.M. to 12 noon on different dates during the months of May and June. Soaked lots were separately exposed to sun's rays on bright and sunny days for 5, 15 and 30 minutes, and for 1, 2, 3, and 4 hours on sheets of cloth in a thin layer. After the above periods of exposure to sun's heat, the various lots were dried in shade in the laboratory room. One soaked lot, on each date, was not at all exposed to the sun and was dried only in the shade. Similarly, another soaked lot, on each date, was dried in the sun from 12 noon till sun-down. Samples without any soaking and exposure to sun's rays were kept for each date to serve as controls.

All the samples treated during any of the three years were stored in the laboratory room till sowing time during the following wheat season. The percentage of loose smut infection, appearing in the month of March during each of the three years, was recorded on the basis of the total number of stools in a plot. The results are set out in Table 1.

The results presented in table 1 show that after a pre-soaking period of 4 hours in water, one hour's exposure to solar heat on bright and sunny days in the months of May and June under conditions obtaining at Ludhiana, which is representative of the Punjab plains, is effective in eliminating completely the internal loose smut infection from seed wheat. Even a 30 minutes' exposure during the month of June in the years 1952 and 1954 has eliminated infection almost completely, whereas the untreated lots show 5.8 to 9.0 per cent infection. During the year 1953, such a brief exposure, though not totally effective, reduced the incidence of infection considerably. In the case of the sample treated on the 11th June, 1953, when the sun's heat was very intense, the infection has been reduced from 16.8 to less than 1 per cent.

It is extremely interesting to note that even a 5 minutes' exposure, which is very brief, is effective in reducing infection remarkably in the case of seed lots treated during the month of June of the years 1952 and 1954.

Even the mere process of soaking seed wheat in ordinary water at room temperature and drying it in shade reduces infection appreciably in the case of all the seed lots experimented upon during the months of May and June of the 3 years 1952, 1953 and 1954 on all the dates.

It may be especially noticed that in the case of seed lots simply soaked in water for 4 hours and then dried in shade only on the 20th May, 11th and 25th June of the year 1953, the incidence of infection has been reduced from 17.0, 16.8 and 19.4 to 12.0, 12.6 and 13.3 percent, respectively. Such reductions in the incidence of infection amount to about 30 per cent and are remarkable. This evidence substantiates the observation made by intelligent farmers that the wheat produce becoming moist as the result of rains, while on the threshing floors, becomes automatically free from loose smut infection to an appreciable extent.

From the results discussed above, it is now evident that for the complete devitalization of the intra-seminal mycelium of the loose smut fungus, *Ustilago tritici*, in the seed wheat soaked in water for 4 hours, a prolonged period of exposure of about 4 hours to solar rays after 12 noon is not necessary. Only one hour's exposure is enough. The rest of the exposure of seed to sun's rays constitutes only the drying process. This result is considered to be of both fundamental and practical importance. No fears with regard to the effectiveness of the solar treatment in the plains of the Punjab during the months of May and June may now be entertained, if soaked seed wheat becomes exposed to the sun's rays for a period of about 1 hour. Subsequent to this exposure, windy or cloudy conditions of the day will not impair the effectiveness of the treatment. The only impor-



TABLE 1. showing the percentage loose smut infection in the treated and untreated samples of seed wheat.

S. No.	Treatment	Percentage loose smut infection in the samples treated during									
		1952 on			1953 on			1954 on			
		9th June	21st June	23rd June	20th May	27th May	11th June	25th June	18th June	20th June	
1.	Soaked seed exposed to the sun for 5 minutes and then dried in shade	3.1	1.5	3.2	Not tried	Not tried	Not tried	Not tried	4.2	4.8	
2.	Soaked seed exposed to the sun for 15 minutes and then dried in shade	1.2	1.5	4.0	8.2	11.9	7.2	15.3	2.0	1.5	
3.	Soaked seed exposed to the sun for 30 minutes and then dried in shade	0	0	0	5.0	9.3	0.7	5.9	0.6	0	
4.	Soaked seed exposed to the sun for 1 hour and then dried in the shade	0	0	0	0	0	0	0	0	0	
6.	Soaked seed exposed to the sun for 3 hours and then dried in shade	0	0	0	0	0	0	0	0	0	
7.	Soaked seed exposed to the sun for 4 hours and then dried in shade	0	0	0	0	0	0	0	0	0	
8.	Soaked seed dried by exposure to solar rays till sunset	0	0	0	0	0	0	0	0	0	
9.	Soaked seed not exposed to the sun at all and dried in shade only	7.7	5.5	4.8	12.0	12.0	12.6	13.3	6.2	8.2	
Percentage smut infection in the untreated samples		8.2	8.2	5.8	17.0	15.2	16.8	19.4	8.5	9.0	

tant point to be kept in mind is to dry the seed thoroughly after the necessary duration of exposure. This is possible in one day, if the day remains bright and hot throughout, or it may require another day or two if cloudy weather supervenes.

Some other points of great practical importance emerge from the results of these experiments.

Since it is the brief period of one hour or so of exposure to solar heat immediately after the soaked seed is spread out to dry on a cloth sheet, or a tarpaulin or a sheet made from Hessian, that is the most potent factor in destroying the internal loose smut infection, maximum advantage of solar heat during this brief period should be taken. This should be done by spreading the soaked seed in a very thin layer. Reports of ineffectiveness of the solar treatment against loose smut had been received by the author in a few instances. On enquiries, it was found that in the case of ineffectiveness of the treatment, the correct procedure had not been followed. In one case, the author himself saw that the soaked seed had been placed in the sun to dry in a layer about one inch thick. The treatment could not be expected to be effective under such a condition, which also impairs the germination of the seed, remaining moist for an unusually prolonged period and being invaded by saprophytic micro-organisms.

Also to augment the effect of sun's heat, the cloth sheet or tarpaulins, etc., should be spread on the ground at about 11.30 A.M., so that they acquire the temperature of the ground by the time the seed is taken out of water at 12 noon for spreading it on them.

Also the ground where the drying sheets or tarpaulins are to be spread, must not be covered with grass or any other vegetation.

#### SUMMARY

It has been shown that after a pre-soaking period of 4 hours in water of seed wheat, only one hour's exposure to solar heat is enough to devitalize the intraseminal mycelium of the loose smut fungus, *Ustilago tritici*. The rest of the exposure to sun's rays constitutes only the drying process.

Even a 5 minute's exposure of soaked seed wheat at 12 noon is quite effective in reducing loose smut infection appreciably.

Even the mere process of soaking seed wheat in ordinary water at room temperature and drying it in shade reduced the amount of loose smut infection by about one-third during the months of May and June of the year 1953.

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## CONTROL OF LOOSE SMUT OF BARLEY WITH SOLAR HEAT IN THE PUNJAB PLAINS

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(Accepted for publication October, 16, 1957)

Loose smut caused by the fungus *Ustilago nuda* (Jens.) Rost., occurs wherever barley is grown. Its incidence, however, varies from place to place and from year to year. In the Punjab State, it is particularly serious in relatively more humid places, like Kangra, Gurdaspur, Ambala and Karnal.

Here, during certain seasons, the incidence of smutted plants may be as high as 15 per cent. As in the case of loose smut of wheat, caused by *Ustilago tritici* (Pers.) Rost., with which it is identical in life-history and morphology of spores, the infected ears are totally transformed into a sooty black powder consisting of the countless spores of the causal fungus. The spores are blown about by wind and infect the developing grains internally. The infected grains ripen like normal un-infected grains. Externally, there is nothing to show that they carry infection within, but when these apparently healthy grains are sown, they give rise at blossoming time only to black smutted heads.

Since this smut is similar to the loose smut of wheat in life-history, theoretically it should be possible to eliminate it by solar treatment, discovered by Professor J. C. Luthra of the Punjab Agricultural College, and Research Institute, Lyallpur (now in Pakistan) to control the latter disease. Solar treatment against loose smut of wheat consists in soaking the infected seed in ordinary water at room temperature for 4 hours from 8 A.M. to 12 noon on any bright and sunny day during the months of May and June and then drying it in the sun in a thin layer on sheets of cloth or on tarpaulins from 12 noon till 4 P.M., or later. Vasudeva and Iyengar (1950) soaked the infected barley seed in cold water from 6 A.M. to 10 A.M., and exposed it to the sun on a brick floor for 7 hours from 10 A.M. to 5 P.M. on the 23rd June, 1949. The treated seed was stored till sowing time during the following wheat season.

The disease was completely controlled by this treatment, which, however, is said to injure the germination of the treated seed slightly. The exact figure for reduction in germination is not given by the authors. Before recommending the large-scale treatment of seed barley with solar heat to eliminate loose smut, and to ascertain exactly its effects on the germination and stand of the resulting crop, it was considered necessary to give the method a trial under conditions obtaining in the Punjab plains.

For this purpose, samples of naturally infected seed barley were treated in May and June, 1952, by soaking them in ordinary water from

8 A.M. to 12 noon (not from 6 A.M. to 10 A.M., the pre-soaking period employed by Vasudeva and Iyengar (1950)). The soaked samples were taken out and spread out in a thin layer on a cloth sheet and exposed to the direct rays of the sun. The samples were dried till sun-set and were stored till sowing time during the following barley season. The treated samples were sown along-side untreated samples during October, 1952. The percentage of smutted stools was recorded in March, 1953, and the data are given in table 1.

TABLE 1. The effect of solar treatment on the incidence of loose smut of barley

S. No.	Particulars of the treated sample	Percentage loose smut infection
1.	Sample No. 1, treated on the 9th June, 1952 at Ludhiana	0
2.	Sample No. 2, treated on the 23rd June, 1952 at Ludhiana	0
3.	Sample No. 3, treated on 20th May, 1952 at Gurdaspur	0
1.	Untreated sample No. 1	3.1
2.	Untreated sample No. 2	1.6
3.	Untreated sample No. 3	5.5

From the data presented in table 1, it is evident that solar treatment of seed barley is effective in eliminating loose smut infection completely both under Ludhiana and Gurdaspur conditions.

The experiment was repeated during the year 1953 at the Plant Pathological Sub-station, Gurdaspur, by using an artificially infected barley sample.

The sample treated with solar heat on the 15th May, 1953 and the untreated sample were sown during the 3rd week of October, 1953. The percentage of smutted stools was recorded in March, 1954. The treated sample gave rise to plants, which were totally free from loose smut. On the other hand, plants from the untreated sample had among them 22.7 per cent smutted plants.

Solar heat during summer months at Gurdaspur, though intense, is milder than at many other places in the Punjab plains. Since the treatment has been completely effective at Gurdaspur, both during 1952 and 1953, no doubts need be entertained regarding its effectiveness in other places in the Punjab plains. Also, as reported by Vasudeva and Iyengar (1950), even the exposure to sun's rays at 10 A.M. of soaked seed barley, is effective in eliminating the smut completely, the solar treatment commencing at 12 noon, as tried by the author, cannot fail to be effective, because of certainly greater intensity of sun's heat at 12 noon than at 10 A.M.



In the trials conducted by the author, no diminution in germination of treated seed of barley T. 4, was observed. In order to ascertain, if this is so in the case of different varieties of barley, 26 varieties and crosses were soaked in ordinary water from 8 A.M. to 12 noon on the 21st June, 1955, which was a very bright and hot day. The soaked seed of different varieties was spread thinly on cloth sheets and was exposed to sun's rays from 12 noon till sunset.

The treated and untreated seed of all the varieties was sown in single rows, repeated three times in different randomised blocks. Counted numbers of seeds of each variety were sown at a uniform depth of 3 inches with a depth-regulating device and the germination was recorded, when it was complete within about a fortnight of sowing, on 3rd Nov., 1955. Data in respect of average percentage germination are given in table 2.

TABLE 2. The effect of solar treatment on the percentage germination of seed of different barley varieties.

S. No.	Barley variety	Average percentage germination	
		Treated seed	Untreated seed
1.	Freja C.I. 7130	81.3	79.3
2.	Pb. T. 5	89.3	90.0
3.	C. 144-6	86.3	88.0
4.	C. 144	87.0	84.0
5.	C. 138-2	90.0	90.0
6.	Hybrid 2	88.7	88.0
7.	Pb. T. 4	92.0	83.0
8.	Excelsior	77.7	78.6
9.	Hybrid 7	90.3	91.3
10.	C. 147	93.7	93.0
11.	Hybrid 3	92.0	92.3
12.	Jet	80.0	81.0
13.	N. P. 13	91.3	92.0
14.	C. 146	94.7	93.3
15.	C. 138	96.3	90.7
16.	Hybrid 5	89.0	92.3
17.	C. 145	94.7	96.3
18.	C. 144	92.0	95.0
19.	N. P. 4	89.7	87.0
20.	No. 124-18	87.3	86.5
21.	N.P. 1	77.3	80.0
22.	C. 124-18 (Second sample)	91.7	91.3
23.	C. 153	92.0	87.0
24.	Lion	88.0	88.0
25.	Ludhiana Local	91.7	92.0
26.	Himalayas	79.0	80.0
27.	Hybrid 4	91.0	90.7
C.D. at 5% : 9.92			
C.D. at 1% : 13.13			
Average percentage germination for the treated series as a whole		88.6	
Average percentage germination for the untreated series as a whole			88.1

The data presented in table 2 show that solar treatment of grains of different barley varieties does not injure their germination even when the exposure of the soaked grains to solar rays is commenced at 12 noon. A slight diminution in germination, as reported by Vasudeva and Iyengar (1950), even when the solar treatment was started at 10 A.M. by them is probably due to the use of a brick-floor, which acquires a higher temperature than the cloth sheets on which the seed was exposed to the sun's heat and dried by the author. In the Punjab State while carrying out the solar treatment on a large scale even against loose smut of wheat, the use of a brick or cement floor is never recommended. The soaked seed is always dried on tarpaulins or on sheets of cloth or Hassian.

As the results reported in this paper have shown not only the effectiveness of solar heat in eliminating loose smut of barley, but also its harmlessness to the viability of the treated seed, the solar heat treatment will be adopted on a large scale in the Punjab State to control this smut also.

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## SOME BIOLOGICAL EFFECTS OF RADIOACTIVE PHOSPHORUS ON PHYCOMYCES BLAKESLEEANUS BURG.

M. R. S. IYENGAR AND P. L. GANJU

(Accepted for publication October 31, 1957)

*Phycomyces blakesleeanus* is known to be most sensitive to radiations and as low a dose as  $\beta$  .001  $\gamma$  can retard its growth (Forssberg, 1943). This organism was, therefore, considered suitable for studies on the biological effects of  $\beta$ -radiations from radioactive phosphorus, the results of which are reported in this paper. The culture in this investigation was a(+) strain No. G. C. 628 of *P. blakesleeanus* obtained from Indian Type Culture Collection.

In preliminary experiments small paper discs were impregnated with neutralised aqueous solution of carrier-free radioactive phosphorus. Different numbers of discs were used to vary the total activity from 0.74  $\mu\text{c}$  to 4.5  $\mu\text{c}$ . Spores were treated on these discs for 48 hours at about 18°C, and then transferred alongwith the discs to 15 ml. of Dextrose-Asparagine-Thiamine medium\*. In the control untreated discs were used. Growth appeared to be normal both in the treated and control series. In another experiment spores were suspended in Dextrose-Asparagine-Thiamine medium into which  $P^{32}$  solution was added to give a total initial activity of about 20  $\mu\text{c}/\text{ml}$ . After 12 days' incubation at 20-2°C. an appreciable retardation in the length of sporangiophores was observed in the treated series as compared to the control. Subcultures made by mass transfers from both the series gave normal cultures, showing thereby that the change induced at this level of activity was only a temporary one, which could not be inherited to the next generation.

In further experiments, washed spores were suspended in neutralised aqueous solution of carrier-free  $P^{32}$  with an initial activity of about 170  $\mu\text{c}/\text{ml}$ , and kept at about 7°C. Aliquots of the suspension were removed at varying intervals, diluted and washed by repeated centrifugation, using aseptic precautions, and then tested for germination and growth. Spores treated for 10 and 20 days were found to have been killed, whereas those treated for 5 days were viable as determined by mass transfers to Potato-Dextrose-agar\*\*. These cultures differed from the untreated controls in that they grew more slowly and one of them produced fewer normal sporangiophores. Most of the sporangiophores were extremely short though fertile (figure 1). The spores from the latter were mostly round as compared to the ellipsoidal shape in the control. Subcultures made by mass transfers from this culture differed from each other. Generally, the

\* Dextrose, 30 gms; Asparagine, 1 gm; Mg So<sub>4</sub> 7H<sub>2</sub>O, 0.5 gms; KH<sub>2</sub>PO<sub>4</sub>, 1.5gms Thiamine, 10 ug/ml; Distilled water, 1000 ml.

\*\* Peeled Potatoes, 250 gms; Dextrose, 20 gms; Agar, Agar 20 gms; Distilled water 1000 ml.



**Fig. 1.** Changes induced by radioactive phosphorus in *Phycomyces blakesleeianus*.

- (a) A culture from treated spores showing fewer normal sporangiophores.
- (b) Control.

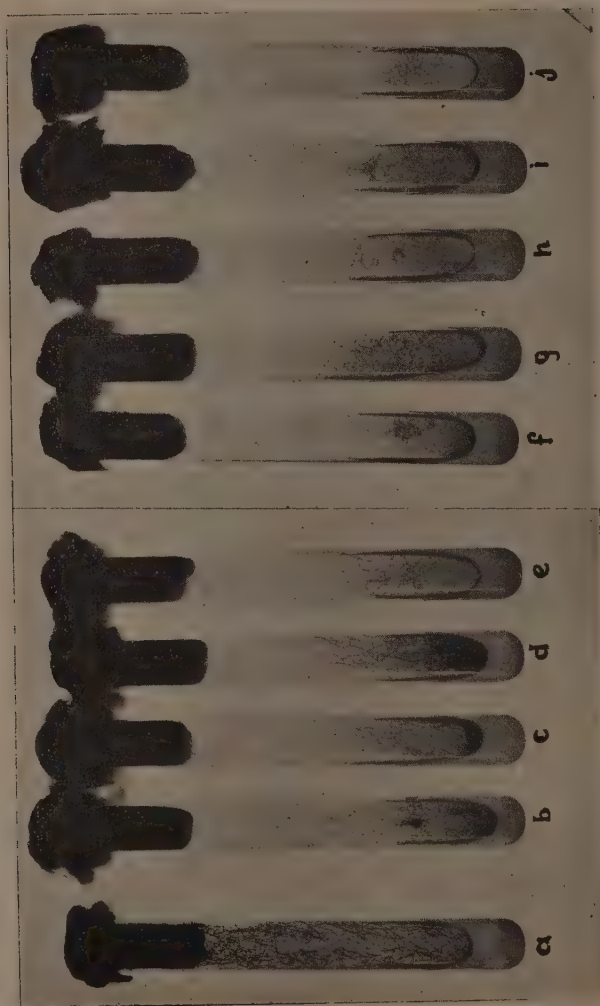


Fig. 2. Variation among single spore cultures in the treated series of *P. blakesleanus* in the third generation.

(a) Control; (b-f) cultures with thinner, shorter and partly sterile sporangio-phores; (g) a culture with abundant diminutive sporangio-phores; (h-j) extremely slow growing cultures with fewer sporangio-phores.



cultures were showing a mixture of the two types of sporangiophores as mentioned above but in one culture the number of short sporangiophores was much higher. Single spore cultures made from the latter differed markedly from the parent and presented a wide range of variation among themselves (figure 2). The variants could be broadly classified into the following types:

1. Slow growing cultures with thinner and shorter sporangiophores as compared to the normal long and broad ones in the control. Some of these sporangiophores were sterile (figure 2, b-f).
2. Slow growing cultures with diminutive sporangiophores measuring only a few millimeters in size (figure 2, g).
3. An extremely slow growing type which produced a few sporangiophores of either of the above types; some of these cultures produced a strong yellow pigment in the mycelium which turned brown with age (figure 2, h-j).

Single spore cultures obtained from one of the extreme forms of type (2), differed from each other in the rate of growth, the extent of pigment formation and other morphological characters. A few non-pigmented cultures among these showed white dot-like accretions of the mycelium macroscopically. Some single spore cultures produced a few normal sporangiophores in addition to the numerous diminutive but fertile ones. A few cultures produced abnormally long and coiling sporangiophores which were sterile. Another interesting form showed branching of the sporangiophores. In most of the cases the sporangiophore branched sympodially, each branch bearing a sporangium at the tip. In some cultures the branching of the sporangiophore was either dichotomous or more than two branches arose from a point (figure 3).

Thus a large number of single spore and mass cultures studied through four successive generations showed that a continuous splitting of characters and apparent reversions to some earlier forms were quite common in the treated series of this fungus. Detailed study of the morphological characters of these variants made for another three generations, however, showed that at least three forms maintained their characters and were stable to some extent. These strains are briefly described below:—

1. Slow growing, mostly mycelial and strongly pigmented;
2. Slow growing, non-pigmented; sporangiophores of two types, one diminutive but fertile with sporangia raised only a few millimeters above the aerial mycelial mat, and the other thinner and shorter than in the control, and mostly sterile; spores, generally round as compared to the ellipsoidal shape in control.
3. Pigmented or non-pigmented, sporangiophores sympodially branched and almost as long as in the control (figure 4).

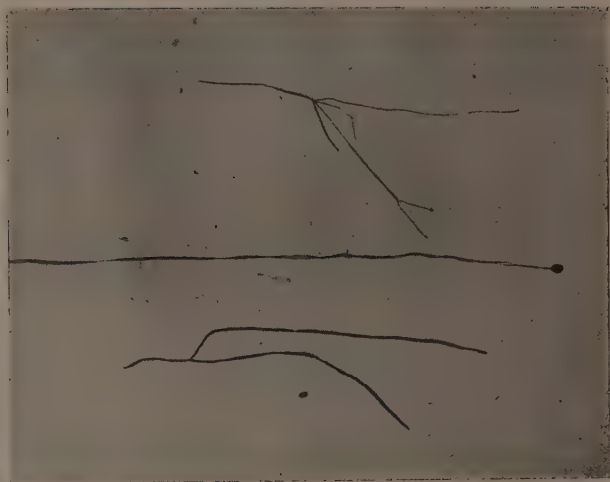


Fig. 3. Variation in branching of sporangiophores of *P. blakesleeae* in the treated series, with a normal fertile sporangiophore in the centre.

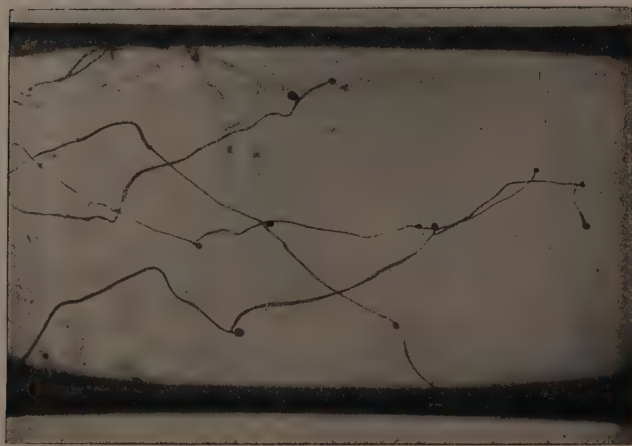


Fig. 4. Sympodially branched sporangiophores of *P. blakesleeae* mutant.

Among the morphological variations, two of the characteristics consistently observed in many cultures, namely the diminutive but fertile sporangiophores and the branched type of sporangiophores were of particular interest in that they cut across the generic criteria established for *Phycomyces*. Long and unbranched sporangiophore has been considered to be such an important character of this genus as to separate it from the other members of Mucoraceae (Zycha, 1935; Bessey, 1950). The sympodial branching of the sporangiophores resembles to some extent with that in *Circinella* and some species of *Mucor*, belonging to the same group. However, it is of interest to note that the two strains, one showing two types of sporangiophores, and the other showing branched ones, could successfully be mated with the complementary (-) strain No. 627 (I.A.R.I.) of *P. blakesleeianus* showing thereby, that the mutants still maintained their sexuality.

In the preliminary studies made so far, mutant strains which did not produce normal sporangiophores on Dextrose-Asparagine-Thiamine medium showed better sporangial formation in this medium, supplemented with other vitamins, which indicate that they are nutritionally deficient mutants and offer considerable promise in the study of morphogenesis in *P. blakesleeianus*.

#### SUMMARY

Spores of *Phycomyces blakesleeianus* were irradiated with P32 at levels ranging from 0.74  $\mu$ c to 170  $\mu$ c/ml. At 20  $\mu$ c/ml temporary dwarfing of sporangiophores was observed while at 170  $\mu$ c/ml. biological and genetical effects were more pronounced. Cultures, in general, tended to be more pigmented, sterile, or produced dwarf but fertile sporangiophores. In some cultures branching of sporangiophores was observed.

Three mutants which showed some stable characters for at least 3 generations have been picked up.

ACKNOWLEDGMENTS: It is a pleasure to express our gratitude to Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology, for his able guidance, valuable criticism and for providing necessary facilities. We are also thankful to Mr. B. S. Bajaj and Mr. M. S. Chatrath for their assistance during the course of work.

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## NOTES ON MISCELLANEOUS INDIAN FUNGI - V

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(Accepted for publication November 25, 1957)

The present paper like the previous ones of the series, gives an account of the new fungi, new host records for the older known ones or those that have not been previously recorded in India. Type specimens of the new species have been deposited at Herb. Crypt. Ind. Orient, I.A.R.I., New Delhi. Series I-IV appeared in Indian Phytopath. Vol. III, 1950, Vol. VIII, 1955, Vol. IX(1) and Vol. IX(2), 1956 respectively.

### 76. *Mycosphaerella murrayae* sp. nov. (Fig. 1).

Maculae amphigenae, circulares, cinereae, marginibus purpureis elevatisque, 1-3 mm. diam. Perithecia amphigena, atra, puncti similia, dispersa per totam partem necroticam maculae, innata, tum erumpentia, subepidermalia, globosa, ostiolata, fusce brunnea, magnit. 90-150  $\mu$  diam. (ut plurimum 110-140  $\mu$ ). Asci hyaline, cylindrici, paraphysati, octospori, 50 - 75 x 10 - 13  $\mu$ . Ascospores dispositae biserialiter in asce, hyalinae, bicellulatae, tenuiter constrictae ad septum, ellipticae vel fuscoideae, apicibus rotundatis, magnit. 13 - 15 x 3 - 5  $\mu$ .

Typus lectus in foliis viventibus *Murrayae exoticae* L. in loco. Dehra Dun die 14 mensis Octobris anni 1955 a J. N. Kapoor.

Spots amphiginous, circular, ash coloured with a raised purple margin, 1 - 3 mm. in diameter; *Perithecia* amphigenous, black, dot-like, scattered throughout the necrotic portion of the spot, innate, later erumpent, subepidermal, globose, ostiolate, dark brown, measuring 90-150  $\mu$  (mostly 110-140  $\mu$ ) in diameter; *Asci* hyaline, cylindric, paraphysate, 8-spored, measuring 50-75 x 10-13  $\mu$ ; *Ascospores* arranged biserially within the ascus, hyaline, bicelled, elliptic to fusoid, ends rounded, slightly constricted at the septum, measuring 13-15 x 3-5  $\mu$ .

On living leaves of *Murraya exotica* L. (Rutaceae) Dehra Dun, 14-10-1955 (J. N. Kapoor).

The diseased spots are very conspicuous on the host. No species of *Mycosphaerella* seems to have been recorded on this host. Three species of *Sphaerella*, namely *S. inflata* Penz., *S. gibbelina* Pass., *S. lageniformis* Rhem., recorded on *Citrus* spp., differ from this in having smaller spores and asci, narrower asci and broader spores respectively.

### 77. *Mycosphaerella pyrina* (E. & E.) Comb. nov.

Syn. *Sphaerella pyrina* E. & E. in North American Pyrenomycetes, p. 275, 1892; Saccardo in Syll. Fung. XI:298, 1895.

On living leaves of *Pyrus communis* L. (Rosaceae), Govt. Gardens, Saharanpur, Uttar Pradesh, 5-10-1955 (J. N. Kapoor).

The perithecia are embedded in leaf tissues and are inconspicuous. These are found scattered in small groups.

78. *Trematosphaeria jasmini* sp. nov. (Fig. 2).

Perithecia dispersa, separata, innata, tandem erumpentia, nigra, carbonacea, globosa, magnit.  $100 - 180 \mu$  (ut plurimum  $150 - 180 \mu$ ) diameter; Asci paraphysati, clavati, basi truncata, magnit.  $70 - 125 \times 10 - 15 \mu$  (ut plurimum  $70 - 90 \times 12 - 14 \mu$ ) Ascospores 8, irregulariter biseriatae, cylindricae vel fusiformes, 6-7 septatae, aliquando constrictae ad medium septum, apicibus rotundatis atque hebetibus, pallide olivaceae, magnit.  $20 - 35 \times 5 - 7 \mu$  (ut plurimum  $28 - 35 \times 5 - 7 \mu$ ).

Typus lectus in ramis arescentibus *Jasmini* sp. in "Catchment area", in loco Simla, die 18 mensis junii anni 1955 a J. N. Kapoor.

*Perithecia* scattered, separate, innate finally erumpent, black carbonaceous, globose, measuring  $100 - 180 \mu$  (mostly  $150 - 180 \mu$ ) in diameter; *Asci* paraphysate, clavate with truncate base,  $70 - 125 \times 10 - 15 \mu$ , (mostly  $70 - 90 \times 12 - 14 \mu$ ); *Ascospores* 8 in each ascus, irregularly biseriate, pale olive, cylindric to fusiform, 5 - 7 septate, sometimes constricted at the middle septum, ends rounded and obtuse, and measure  $20 - 35 \times 5 - 7 \mu$  (mostly  $28 - 35 \times 5 - 7 \mu$ ).

On drying up twigs of *Jasminum* sp., Catchment area, Simla 18-6-1955 (J. N. Kapoor).

The fungus was collected from a drying *Jasminum* plant and was very prominent on account of its shining black perithecia, which are scattered throughout the dead areas of the twigs.

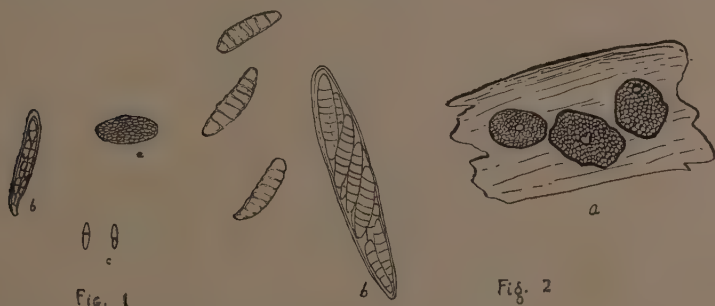


Fig. 1. *Mycosphaerella murrayae*  
(a) Perithecium (b) Ascus (c) Ascospores

Fig. 2. *Trematosphaeria jasmini*  
(a) Perithecia (b) Ascus (c) Ascospores



79. *Massarina psidii* sp. nov. (Fig. 3)

*Perithecia* separata, dispersa, innata, tandem erumpentia, globosa vel subglobosa, ostiolata, membranacea, fuscae brunnea, magnit. 200–230  $\mu$  diam. *Asci* hyaline, aparaphysati, cylindrici vel clavati, crassi ad apicem, octospori, magnit. 70 – 105 x 14– 17  $\mu$ . *Ascosporae* hyalinae, biseriatae, ellipticae vel oblongae, apicibus rotundatis atque hebetibus, 3-septate, haud constrictae ad septum, circumdatae vagina tenui, hyalina atque mucosa, magnit. 14 – 20 x 4 – 6  $\mu$ .

Typus lectus in ramulis arescentibus *Psidium guajavae* L. ad Azadpur, Delhi die 1 mensis octobris anni 1955 a J. N. Kapoor.

*Perithecia* separate, scattered, innate later erumpent, globose or subglobose, ostiolate, membranous, dark brown and measure 200–230 diameter; *Asci* aparaphysate, cylindric to clavate, broadened at the apex, 8-spored and measure 70 – 105 x 14 – 17  $\mu$  in size ; *Ascospores* hyaline, biseriate, elliptic to oblong, ends rounded and obtuse, 3-septate, not constricted at the septum, surrounded by a thin, hyaline, mucous sheath and measure 14 – 20 x 4 – 6  $\mu$ .

On drying twigs of *Psidium guajavae* L. (Myrtaceae), Azadpur, Delhi, 1–10–1955 ( J. N. Kapoor).

This fungus was observed on the twigs, bearing scabby fruits. Whether it is the cause of that particular scab disease, is not yet determined. A few scattered, erumpent, dot-like or lens shaped perithecia appeared on the discoloured areas which covered large surface of the drying up twigs. A thin hyaline mucous sheath round the spores was clearly visible, when those were mounted in water, but it was indistinct, when the spores were examined in lactophenol mount.

80. *Pringshiemia oldenlandiae* sp. nov. (Fig. 4).

*Perithecia* puncti similia, subepidermalia, erumpentia, plurima, globosa vel subglobosa, ostiolata, glabra, fuscae brunnea, parietibus crassis, magnit. 120 – 150  $\mu$  diam. *Asci* plurimi, hyalini, obovati, sessiles, aparaphysati, octospori, magnit. 60 – 70 x 35 – 40  $\mu$ . *Ascosporae* hyalinae, cylindricae vel aliquantum angustatae infra, apicibus rotundatis, muriformes, ornatae 3 – 4 septis transversalibus atque 2 – 3 septis longitudinalibus, rare constrictae ad septum medium, magnit. 28 – 32 x 10–12  $\mu$ .

Typus lectus in ramulis emortuis *Oldenlandiae* sp. ad Badnapur, in Hyderabad, die 30 mensis novembris anni 1955, a N. R. Yardi.

*Perithecia* dot-like, subepidermal, erumpent, numerous, globose or subglobose, ostiolate, glabrous, dark brown, measuring 120 – 150  $\mu$  in diameter with a thick wall; *Asci* numerous, hyaline, obovate, sessile, 60–70 x 35 – 40  $\mu$ , 8-spored, aparaphysate; *Ascospores* cylindric or somewhat narrowed below, both ends rounded, hyaline, multiseptate, with 3 – 4 cross septa and 2 – 3 longitudinal septa, rarely once constricted at the middle septum and measure 28 – 32 x 10 – 12  $\mu$ .

On dead twigs of *Oldenlandia* sp. Badnapur, Hyderabad, 30-11-1955 (N. R. Yardi).

81. *Pseudopeziza radians* (Rob. et Desm.) Sacc. in Syll. Fung., 8 : 724, 1889.

On living leaves of *Campanula colorata*, near Batote, Kashmir State, 20-10-1955 (R. L. Munjal).

The black, minute, almost superficial, disc-like *apothecia* were found scattered on the upper leaves, which also showed infection of rust, *Coleosporium campanulae*. The *asci* are paraphysate, clavate and measure  $60-65 \times 6-8 \mu$ . Paraphyses are copious, hyaline and filiform. *Ascospores* are hyaline, ovoid-elliptic, uniseriate and measure  $9-11 \times 3-4 \mu$ .

82. *Phomopsis cacti* Grove in Kew Bull. p. 54, 1917

Syn. *Phoma cacti* Berk. Sacc. in Syll. Fung., 3 : 138, 1884.

On dead stems of *Opuntia* sp. Manali, Kulu division, Punjab, July 1955, (L. M. Joshi).

Black dot-like pycnidia appear on the ashy coloured spots. These contain both alpha and beta spores, though the latter were found in a few pycnidia only. The alpha spores are elliptic, single celled and measure  $6-8 \times 3-3.5 \mu$ , while the beta spores are hooked, hyaline,  $12-18 \times 1-1.5 \mu$ .

83. *Cytospora rhoïa* Fr., in Syst. Myc., 2 : 546; Sacc. in Syll. Fung. 3 : 257, 1884.

On dead leaves of *Mangifera indica* L. (Anacardiaceae), Poona, 27-2-1949 (R. L. Munjal).

There is no record of any *Cytospora* species on this host. The species listed above agrees closely with *C. rhoïa* Fr. which is known to occur on leaves and branches of *Rhus* sp., a member of the same host family, Anacardiaceae.



Fig. 3

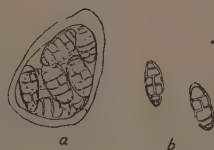


Fig. 4

Fig. 3. *Massarina psidii* (a) Ascus (b) Ascospores

Fig. 4. *Pringshiemia oldenlandiae* (a) Ascus (b) Ascospores

84. *Sphaeropsis loranthi* sp. nov. (Fig. 5)

*Pycnidia* immersa, tum erumpentia, separata, subglobosa, pillata, crassis parietibus praedita, nigra, carbonacea, ostiolata, magnit. 60–80  $\mu$ . diam. Conidiophori conspicui, hyalini, tenues ad 70  $\mu$  longi, Sporae, oblongo-ovoideae, atrobrunneae, unicellulae, magnit 35 – 50 x 16–20  $\mu$ .

Typus lectus in foliis *Loranthi* sp. parasitici *Platanus orientale* in Kangan, Indus Valley in regione Kashmir, die 14 mensis Octobris anni 1955 a R. L. Munjal.

*Pycnidia* innate, then erumpent, separate, subglobose, papillate, thick walled, black, carbonaceous, ostiolate, measuring 60 – 80  $\mu$  in diameter. *Conidiophores* conspicuous, hyaline, slender, upto 70  $\mu$  long and bearing spores singly at their tips; spores oblong-ovoid, dark brown, one celled, measuring 35 – 50 x 16 – 20  $\mu$ .

On living but fallen leaves of *Loranthus* sp. parasitising *Platanus orientale*, Kangan, Indus Valley, Kashmir, 14–10–1955 (R. L. Munjal).

85. *Ascochyta chenopodii* Rostr. in Bot. Tidsskr. 26: 311. 1905, Died. in Ann. Mycol. 10 : 139, 1912; Sacc. in Syll. Fung. 8 : 345, 1906.

On living leaves of *Chenopodium album* I. A. R. I., New Delhi, 2–3–1938 (A. Khan).

*Pycnidia* are formed in brown coloured round or irregular spots, mostly on the upper surface. Spores are hyaline, elliptic to cylindric, at first one-celled but later become two-celled and slightly constricted at the septum.

86. *Septoria arisaemae* Petch in Annals R. Bot. Gard. Peradeniya, 5 (3) 236, 1917; Sacc. in Syll. Fung., 25 : 406, 1931.

On living leaves of *Arisaema* sp. Mussoorie, 10–10–1955 (J. N. Kapoor).

The infection was rare, only a few leaves were found to be diseased. The spots are irregular to circular with yellowish halo. *Pycnidia* are amphigenous, dark brown, globose to sub-globose, subepidermal and 35 – 70  $\mu$  in diameter. Spores are hyaline, slender, sinuous, 1–3 septate and measure 20 – 40 x 1.5 – 2  $\mu$ .

87. *Septoria erianthi* sp. nov. (Fig. 6)

Maculae amphigenae, lineares, alutaceae, magnit. 10 – 25 x 1.5 mm. *Pycnidia* amphigena, disposita in unam lineam per longitudinem maculae, atrobrunnea, subepidermalia subglobosa, ostiolata, ostiolis ad 18  $\mu$ . diam., magnit. 120 – 150 x 90 – 110  $\mu$ . Sporae hyalinae, tenues, sinuosae, 2–5 septatae, magnit. 30 – 60 x 1.5 – 2  $\mu$ ., insidentes cellulis ellipticis brevius exparietibus pycnidialibus oriuntur.

Typus lectus in foliis viventibus *Erianthi munjae* Roxb. ad Azadpur, Delhi, die 1 mensis octobris 1955, a J. N. Kapoor.

*Spots* amphigenous, linear, tan coloured and measure  $10-25 \times 1-5$  mm. in size. *Pycnidia* amphigenous, arranged in a row along the length of the spot, dark brown, subepidermal, subglobose, ostiolate (ostiole upto  $18 \mu$  in diameter) and measuring  $120-150 \times 90-110 \mu$ ; *spores* hyaline, slender, sinuous, 2-5 septate, measuring  $30-60 \times 1.5-2 \mu$ , borne on short elliptic cells of the inner pycnidial wall.

On living leaves of *Erianthus munja* Azadpur, Delhi 1-10-1955 (J. N. Kapoor).

88. *Septoria pimpinellae* Laubert in Centraalbl. f. Bakater. Paras. etc. II Abt. 52, p. 242, 1920; Sacc. in Syll. Fung., 25 : 456, 1931.

On living leaves of *Pimpinella diversifolia* DC., Mussoorie, 10-10-1955 (J. N. Kapoor).

Numerous reddish angular *spots* were observed on the leaves; *Pycnidia* are black, dot-like, and measure  $50-90 \mu$  in diameter. *Spores* are hyaline, acicular, slightly curved, 1-5 septate and  $25-45 \times 1-1.5 \mu$ .



Fig. 5

Fig. 5. *Sphaeropsis loranthi*  
(a) T. S. through Pycnidium (b) Conidia

Fig. 6

Fig. 6. *Septoria erianthi*  
T. S. through leaf showing pycnidium

89. *Septoria saniculina* sp. nov. (Fig. 7)

Foliorum maculae albae vel alutaceae, amphigenae, irregulariter circulares, marginibus purpureis elevatisque, nervis circumscriptae, magnit. 1-4 mm. diam. Pycnidia amphigena, subepidermalia, atrobrunnea, globosa vel subglobosa, ostiolata, magnit.  $45-140 \mu$  diam. (ut plurimum  $90-120 \mu$ ). Spores tenues, filiformes, hyalinae, 1-5 septatae, apicibus acutis, magnit.  $24-53 \times 1.5 \mu$  (ut plurimum  $45-50 \times 1.5 \mu$ ).

Typus lectus in foliis viventibus *Saniculae europae* L. in loco Mussoorie, die 10 mensis octobris 1955, a J. N. Kapoor.

*Spots* amphigenous, tan coloured with a raised purple margin, irregularly circular, delimited by veins and measuring 1–4 mm. in size. *Pycnidia* dark brown, globose to subglobose, subepidermal, ostiolate, measuring 45–140  $\mu$  in diameter (mostly 90–120  $\mu$ ); *Spores* slender, sinuous, hyaline, 1–5 septate, tips acute, 24–53 x 1.5  $\mu$  (mostly 45–50 x 1.5  $\mu$ ).

On living leaves of *Sanicula-europaea* L., Mussoorie, 10–10–1955 (J. N. Kapoor).

*Septoria saniculae* E. & E. has been recorded on this host genus (Sacc., 10: 466) but it differs from the fungus described above in having smaller size of spots as well as spores (20 x 1  $\mu$ ).

90. *Discosia artocreas* (Tode) Fr. in Summ. veg. Sc. p. 423; Sacc. in Syll. Fung. 3 : 653, 1884.

On dead and fallen leaves of *Pyrus communis* L. (Rosaceae), Maharaja's Garden, Srinagar, 19–10–1955 (R. L. Munjal).

91. *Scolecotrichum gei* sp. nov.

Maculae amphigenae, circulares vel angulares, circumdatae nervis, brunneae marginibus atrobrunneis elevatisque, magnit. 2–5 mm. Fructificationes amphigenae sed praecipue in pagina inferiore foliorum. Stromata parva, gregaria, in medio macularum subepidermalia, postea erumpentia. Conodiophori laxi fasciculati, saepe densi, olivacei, haud ramosi, haud septati, geniculati magnit. 18–30 x 3–3.5  $\mu$ . Conidia olivaceo-brunnea, cylindrica vel oblonga, nonnulla ad apices fastigata, apicibus rotundatis, bicellulata, non constricta ad septum magnit. 10–18 x 3–4  $\mu$ . (ut plurimum 14–17 x 3.3.5  $\mu$ ).

Typus lectus in foliis viventibus *Gei urbani* L. in "Catchment area", a Simla, die 18 mensis octobris 1955, a J. N. Kapoor.

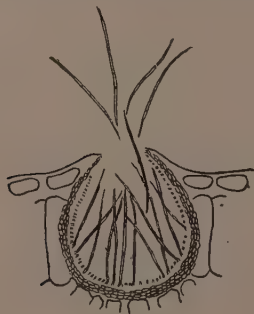


Fig. 7

Fig. 7. *Septoria saniculina*  
T. S. through leaf showing pycnidium



Fig. 8

Fig. 8. *Scolecotrichum gei*  
(a) Stroma with conidiophores (b) Conidia.



*Spots*, amphigenous, circular to angular, delimited by veins, brown coloured with dark brown raised margins and measure 2 – 5 mm. in diameter. Fruiting amphigenous but chiefly on lower leaf surface. *Stromata* small, dot-like, gregarious, in the centre of the spots, subepidermal, later erumpent and effuse. *Conidiophores* in loose fascicles, often dense, olivaceous, unbranched, unseptate, geniculate, and measure 18 – 30 x 3 – 3.5  $\mu$ . *Conidia* olive brown, cylindric to oblong, a few slightly tapering towards the tips, ends rounded, bicelled, not constricted at the septum and measure 10 – 18 x 3 – 4  $\mu$  (mostly 14 – 17 x 3 – 3.5  $\mu$ ).

On living leaves of *Geum urbanatum* L. Catchment area, Simla 18–6–1955 (J. N. Kapoor).

92. *Ramularia decipiens* Ell. & Ev. in Jour. Mycol., 1 : 70; Saccardo in Syll. Fung. 4 : 215, 1886.

On living leaves of *Ranunculus scleratus* L. (Ranunculaceae), in the fields opposite dak bangalow, kud (Kashmir State), 3–10–1955 (R.L. Munjal).

The leaves show whitish to slightly dirty, powdery growth in angular leaf spots which are delimited by veins. These later turn tan coloured. The conidiophores are in clusters and measure 35 – 70 x 3 – 5  $\mu$ ; *Conidia* are hyaline, 1–3 septate, constricted at the septa, cylindric and measure 20 – 35 x 5 – 8  $\mu$ . Sometimes some ovate spores are also found, which give the impression that the fungus lies more close to *Ovularia*.

93. *Helicotrichum obscurum* (Corda) Sacc. in Syll. Fung. 4 : 313, 1886.

On dead leaves of *Pyrus malus* L. (Rosaceae), Maharaja's Garden, Srinagar, 18–10–1955 (R. L. Munjal).

The fungus forms dark brown velvety cushion like growth on the leaves, which is chiefly due to the aggregation of sterile hyphae, apparently to protect the spores. Sterile hyphae are erect, dark brown, verrucose with helicoid ends, *Conidia* are hyaline, one celled, fusiform, sometimes approaching falcate, ends, acute and measure 8 – 18 x 2  $\mu$ , borne acrogenously on simple, short, hyaline conidiophores.

94. *Hadrotrichum phragmitis* Fuckel in Symb. Mycol., p. 221, 1869; Hoehn. in Syst. Fung. Imperfecti, p. 349, 1923.

On stem, leaf and leaf-sheath of *Phragmitis* sp., (Gramineae), Hindan Nadi, Delhi, 24–12–1954 (G. Lal).

The fungus forms black elongated and erumpent sporodochia, which apparently look like telia of some rusts. *Conidiophores* are compact, dark brown, continuous, apex obtuse and bear globose, single celled *conidia* at their tips, which measure 8–12  $\mu$  in diameter.

Our sincere thanks are due to Dr. R.S. Vasudeva, Head of the Division of Mycology and Plant Pathology, for his keen interest, helpful criticism, encouragement and providing necessary facilities for this work. We are also greatly indebted to Rev. Father Dr. H. Santapau, Head of the Botany Department, St. Xavier's College, Bombay for rendering latin diagnosis of the new species.

Division of Mycology and Plant Pathology,  
Indian Agricultural Research Institute, New Delhi.

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## Phytopathological Notes

**A new physiologic race of brown rust on wheat in India:**  
 R. S. VASUDEVA, V. C. LELE AND D. P. MISRA. A new physiologic race of *Puccinia triticina* Eriks., not hitherto recorded from India, has been picked up from the *Rabi* crop of 1952-53. This collection from Punjab contained three races 10, 20 and 107 besides the new race. The first indication of the new race was obtained on Agra local wheat, which is known to contain a mixture of types of *Triticum vulgare*. Agra local wheat has long been used as a control being susceptible to all the known Indian races of the three rusts of wheat. The newly isolated race however clearly shows well-marked susceptibility as well as resistance in several stocks of Agra local on different leaves. The new race appears to resemble closely with race 70 which has been first reported by Roberts (1936)\* from Wales, United Kingdom. For comparative purposes the reactions of the differential hosts to the newly isolated race of *Puccinia triticina* Eriks. and that of race 70 and 10 are set out in Table below:

Race	Malakof	Carina	Brevit	Webster	Leros	Mediterranean	Hussar	Democrat
New race	X	4	4	4	4	0;-1	X	0;-1
70	2-4	3-4	3-4	3	3-4	0;-1	0;-2	0;-2
10	4	4	4	4	4	1-2	2-3	1-2

For its slight resemblance to race 10, this race was tested along with the former on 41 other wheats comprising different species of *Triticum* (*T. compactum*, *T. durum*, *T. monococcum*, *T. dicoccum* and *T. vulgare*), 10 exotics, 9 New Pusa and 1 Kanpur improved variety. Unlike race 10, to which varieties E.671 and NP 814 showed susceptibility, the newly isolated race produced resistant type of infection (0;-1) on these two varieties, clearly differentiating it from race 10.

Whereas stable differential Malakof shows clear and consistent susceptibility to race 10 under all conditions, the new race shows increasing resistance (type 2 to 0;) under higher temperatures (50-85°F) and bright weather, and increasing susceptibility (3-4) under lower temperatures (35-60°F) and poor light. Resistant type of infection on Agra local always yielded the new race, whereas susceptible type of pustules yielded a mixture of races as earlier indicated. Need of changing Agra local as a standard susceptible variety appears desirable.

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\*Roberts, F. M. (1936) Ann. Appl. Biol., 23, 2: 271-301.

A new physiologic race of *puccinia graminis tritici* (pers.) **erikss. and henn. in India:** R. S. VASUDEVA, V. C. LELE AND M. H. RAO  
 A new physiologic race of black rust of wheat, *Puccinia graminis tritici* not hitherto recorded from India\* has been picked up from Lahaul Valley (Punjab) from 1954 crop. The collection contained, besides a new race, races 21 and 40, which are found to be most common in recent years. The types of infection produced by the new race on the differential hosts are given below:-

Place of collection	Little Club	Marquis	Reliance	Kota	Arnavtika	Mindum	Spelmar	Kubanka	Acme	Einkorn	Vernal	Khapli
Bargual	4	4	0;	4	4	4	4	4	3-4	3-4	0;-1	0;-1

This race differs from race 21 by the susceptible type of infection produced on Einkorn. It resembles race 17 of the International Register of Black Rust Races.

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 Indian Agricultural Research Institute, New Delhi.

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## BOOK REVIEW

STAKMAN, E. C. & J. GEORGE HARRAR, **Principles of Plant Pathology**. The Ronald Press Company, New York, 1957. \$ 8.00.

The Ronald Press Company, New York, has published in August, 1957, a book, 'Principles of Plant Pathology', written by two eminent American Scientists, Dr. Elvin Charles Stakman, Professor Emeritus of the University of Minnesota, and Dr. J. George Harrar, Director of Agriculture, Rockefeller Foundation. This book of 581 pages is very comprehensive and authoritative and takes into account the researches of no less than 785 plant pathologists, mycologists and agricultural scientists of the world. Work of several Indian plant pathologists has been profusely cited in the text.

The book is divided into 18 Chapters in which in logical sequence are dealt: 1. The Importance of Plants, 2. The Importance of Plant Diseases, 3. The Nature and Classification of Plant Diseases, 4. Causes of Plant Diseases, 5. The Nature and Classification of Plant Pathogens, 6. Growth and Reproduction of Plant Pathogens, 7. The Genetics of Plant Pathogens, 8. The Production and Liberation of Inoculum, 9. The Dissemination of Plant Pathogens, 10. The Phenomena of Infection, 11. The Effect of Environment and Nutrition on Disease Development, 12. Plant Diseases of International Importance, 13. Diseases in Transit and Storage, 14. Quarantine, Eradication Campaigns, and International Plant Protection, 15. Cultural Practices in Disease Control, 16. Chemical Control, 17. Resistant Varieties, and 18. Future Problems and Prospects. At the end, in three Appendices, are given a list of Important Books in Plant Pathology, and of Principal Pathogens and Insects cited in Text.

From this Table of Contents it would be obvious that the subject matter has been dealt with in great detail. The authors are experienced teachers, and as such, write from personal knowledge of the problems that confront the teacher as well as the student in the study of plant pathogens. In these days of world-wide food shortage 'unless plant diseases are contained, the world's efforts to feed itself cannot be successful. Every loss from disease reduces the average yield per unit of cultivated area, and these losses increase the pressure upon the land available for agricultural production and make even more acute the problem of feeding a growing world population'. The book deserves careful study as it illustrates very convincingly the ecologic and physiologic relationship between host and pathogen, the nature and development of epidemics, and with the international aspects of plant disease control. As stated by the authors in the Preface, their emphasis is on the pathogens which afflict food producing plants because of the importance of these plants to society and to the growing demand for increased production of basic foods throughout the world.

In Chapter 6, the authors have discussed in great details the factors which govern growth and reproduction in plant pathogens because the



success of these organisms in struggle for existence depends largely on their ability to grow and reproduce. "On what can a plant pathogen grow and reproduce? Under what environmental conditions can it grow and reproduce? How fast can it grow and reproduce? How well can it survive unfavourable conditions? How fast and how far can it be disseminated? These primary questions must be answered with respect to each of many thousand plant pathogens. The answers are different for each one, and they are likely to change because the pathogens themselves can change. A complete set of answers for any individual pathogen would require complete knowledge regarding its physiology, ecology and genetics". As the authors have stated "there is considerable knowledge about *how* many of them behave but very little *why* they behave as they do". The book contains representative examples of the behaviour of plant pathogens, viruses, bacteria and fungi, under diverse conditions. In addition to so many other's, Bedi's work on *Sclerotinia sclerotiorum* deserves special mention. It has been extensively quoted as illustrative of the relative importance of genetic factors and environment in the growth of the Punjab race and the Canadian mutant, and in their capacity to produce functional sclerotia and normal apothecia.

The chapter dealing with 'The Genetics of Plant Pathogens' describes the phenomenon of inheritance and variation in these organisms. This has been illustrated by numerous examples, but '*Puccinia graminis* var. *tritici*' has been discussed in detail because it illustrates so clearly the importance and difficulties of classifying biotypes within a taxonomic category of a pathogen'. Who could have written on this subject better than Dr. Stakman himself, the originator of the concept of Physiologic Races? Every plant pathologist and plant breeder now recognises the absolute necessity of possessing a full knowledge of physiologic race flora for successfully breeding disease resistant varieties. Under 'Dissemination of Plant Pathogens' it has been shown how spores can travel over long distance with wind currents in absolute defiance of political boundaries to cause epidemics in distant lands. In the case of such pathogens, knowledge of foci of infection is a great aid in their control because it is obviously easier to put out of action inoculum in a limited area initially at the source than at a later stage when the pathogen has multiplied its strength a billion-fold and spread over huge areas.

Although K. C. Mehta's earlier papers on cereal rusts in India have been cited, his work on 'Dissemination in Relation to Initial Rust Outbreaks' published as Scientific Monograph No. 18 of Indian Council of Agricultural Research, 1952, 368 pp., has probably escaped the notice of the authors. With the help of 11,355 wind-trajectories, and of dates of spore-showers and rust appearance, Mehta was able to locate important foci of infection, and suggest control of epidemics by direct means.

It is recognised that the cultivation of disease - resistant varieties is by far the best and least expensive of all other control methods. the breeding of such varieties has its own limitations. 'One of the most difficult problem is to maintain the continuity of resistant varieties when the population of physiologic races are shifting, as is conspicuously true of wheat stem rust and certain other rusts'. Consequently, other methods

too must be sought. In recent years, emphasis on Chemical control has revived and the reader will find a perusal of that chapter very instructive. As an example of what eradication of alternate host can do to control a disease, the case of barberry eradication in United States has been cited. Nearly a half-billion barberry bushes have been destroyed, and it is calculated that the campaign has reduced annual losses from stem rust in the United States from an average of 40 million bushels to less than 15 million bushels, resulting in annual savings to farmers of well over 30 million dollars.

In Chapter 18, the authors have given a resume of our Past Progress, Present Problems and Future Prospects, and have correctly said that 'There will be far greater opportunity in future for fundamental researches on life process than ever before'. 'An effort is made to place the problem of plant disease control in the proper setting, bring out the International aspects of the science, and suggest methods by which world progress might be made toward the solution of some of these problems which are vital to all mankind'.

There can be no two opinions about the very great importance of the book, as a text for post-graduate students specializing in Plant Pathology, and as a reference manual for research workers and specialists, praise the authors as well as the publishers deserve to be congratulated on this excellent production.

R. PRASADA





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Conovor. R.A. (1948).....Studies of two viruses causing mosaic disease of soyabean. *Phytopathology*, 38 : 724-735.

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